

Social security for students

The dependence of British students on schemes to help the indigent is a sign of sickness in British higher education. The remedy should be radical.

UNIVERSITIES are nothing without students, but in most places operate in a sellers' market and are over full. Not so in impoverished Britain, where an elaborate system for meeting part of students' maintenance costs out of public funds is once again to be scrutinized by a group of civil servants and government politicians bent on removing anomalies and cutting costs. The new inquiry, announced last week, will be the third since the present government came to office in 1979. But this time, the government seems in no particular hurry. The new Secretary of State for Education and Science, Mr Kenneth Baker, said last week that he hoped the inquiry will be completed this year, but that it will be followed by a period of consultation. A conclusion, if any, will certainly be too late for the academic year 1987-88, that in which the next British general election must at the latest be held, which may be part of the government's calculation.

Students' grants have been a nettle too painful to grasp for more than a decade, since the Treasury first told the Callaghan government that Britain could no longer afford them. The new inquiry, like its predecessors, is a damage-control device, probably not the last that Baker will have to cobble together, but the need for it is nevertheless a telling proof of the impoverishment of British higher education. The origin of the present scheme was the liberal doctrine thirty years ago that young people able to win places in higher education should not be dissuaded by cost from taking advantage of them. So local authorities were required to pay the tuition fees of British students at British universities, polytechnics and colleges and also to provide them with a maintenance grant, originally just about enough to keep body and soul together and to buy an occasional book.

Over three decades, two trends have been at work: the government has sought to cheapen the cost of keeping students by stiffening the rules by which grants to individuals with well-off parents are reduced, while ingenious students have taken to using the British system of social security to top up their incomes, claiming unemployment pay in the summer vacations, and the bread-line payments called supplementary benefits and housing allowance then and at other times. British governments always boast, as Baker did last week, that the system is the "most generous in the world", which may be true. The system is also among the most cockeyed, which is why it should be changed.

Cover-up

To what? Last week's inquiry is a cover-up for the government's defeat over a mean-minded plan to trim students' entitlement to social security benefits by disqualifying them from housing benefit, entitlement to which is independent of parental income. Two months ago, the government was warned by its own advisory committee on the social services that it would probably be illegal to exclude just one class of claimants from a system of social benefits whose equitable operation requires non-discriminatory rules. Now the government has backed down, and Baker, with plenty on his plate already, has to pick up the pieces. He is prudent not to hurry, for this is the seamy side of British higher education.

The cost of student maintenance, £2,800 million a year, is more than the £2,000 million public subvention of British

universities through running costs and tuition fees, which cater for roughly a third of all students. One of the reasons why the government six years ago embarked on a reduction of the number of university students was its fear that maintenance grants were a blank cheque on its dwindling tax revenues. Yet the standard grant (£1,800 a year outside London and Oxbridge) is not enough to keep a young adult reasonably housed and fed, let alone read, while for most students the social security system exploited to the limit will provide only a few hundred extra pounds a year.

The result is that everybody is soured by the problem of student grants. The government is over a barrel, and resents it, while many of its supporters are forever asking why students should be paid to enjoy the privilege of higher education. Students feel impoverished (which they are) and pauperized (which they need not be), and resent that. The universities wring their hands most often about their own impoverishment (four per cent down in real terms next academic year), but extend their self-pity to embrace their students in the sure knowledge that there is nothing they can do about the students' plight and, perhaps, because another bout of student trouble might be the last straw. These are the real reasons why the system needs overhauling.

Solution

Here is how to go about the job. First, whatever government supporters say, there is a need in Britain for a mechanism that will help young people to get themselves a higher education. Britain, which prides itself on being a place that must live by its trained wits, is already desperately short of modern skills, partly because the participation rate among the able children of poorer families is too low. That should be recognized at the outset. But, second, present arrangements do commit the British government to writing blank cheques that it may not be able to afford, whose purposes it does not understand, and whose total amount can be contained only by restricting the total amount of higher education on offer, which is in nobody's interest.

So why not trade the 1950s principle of a grant for every would-be student who can find a place in higher education for the less generous but more realistic and equitable principle that grants will be awarded either on the basis of merit or of national need, but will no longer be mandatory on the government through its proxies, the local education authorities? The short answer is that the shift would trigger a tremendous row. Better that than an endless fudge.

If the government has the stomach for the fight, the outcome will not only put a cap on the cost of higher education but could be immensely beneficial for British higher education, which now differs from that in most other countries not merely in the narrowness of the courses which it offers, and in the pre-shrinking specialization it requires of intending students, but in its almost total lack of scholarship funds. But experience elsewhere shows that educational institutions are both more economical and more humane in their administration of student aid than a central bureaucracy can hope to be. So a large chunk of what the British government now spends on student aid could



usefully be transferred to institutions for them to spend instead. By making deals with individual students about repayment in kind for financial help, to which end they would have an incentive for the first time, much better use would be made of the resources spent on student aid and more students would be helped. So why not give that a try?

But no more than half of what the government spends should be administered by the universities, polytechnics and colleges, for too much power over the fates of students would give them the illusion that their individual sizes had been fixed for good. That is why the greater part of present government aid for students should be spent quite differently, on individual student scholarships that individual students may take to the institutions of their choice. Together, these two components of a different system could contain the present cost of student aid, make more efficient use of what is spent, restore a measure of autonomy to higher educational institutions which have already lost too much of it and restore a sense of independence to students, who cannot wholly welcome the idea of beginning adult life as clients of a creaking system of social benefits.

There are several snags, not the least of which is the government's own predilection towards the notion that business, which benefits from such skill as British higher education engenders, should step in to help, by means of sponsorship schemes for potential employees. This was the general cry when Baker announced his new inquiry in the House of Commons last week. But a scheme that puts students in the pockets of future employers is no better than one that makes them depend on social security. So why not student loans? The Prime Minister's promise that grants would be replaced by loans has twice been defeated by the Treasury's calculation that the short-term cost would be increased, but that objection applies only if the whole of student aid at present is shifted to the banks. There is, however, a need for assistance at the margin by way of loans for students whose opinions of the benefits of higher education to themselves may differ from those of the authorities, as well as for those who seek a second change or a period of retraining. Why should not Britain follow a dozen other countries by making a modest beginning with loans for people in such special circumstances? □

Selling lamb short

The British government's decision to ban the sale of some lambs may yet backfire.

THE British Ministry of Agriculture, Fisheries and Food seems to have had a bad Chernobyl. In the days immediately after the tragic accident in the Ukraine, there was a general complaint that too little had been done too late to monitor the occurrence of radionuclides in British food. Matters were not much helped by the appearance of the Secretary of State, Mr Michael Jopling, on a television programme in the course of which it became clear that he had not previously heard of the notion that doses of radiation smaller than the accepted safety limits could nevertheless be dangerous. Now the ministry has swung from complacency to the other extreme, and has banned for 21 days from last Friday the sale of lamb from North Wales and Cumbria. The decision seems to have been taken on the strength of 100 measurements of ^{137}Cs in lambs born earlier this season, 19 of which showed more than 600 Bq kg^{-1} of total caesium among which were 9 exceeding $1,000 \text{ Bq kg}^{-1}$. The ministry, not now particularly popular among hill farmers for its expressed (and reasonable) wish to reduce the subsidies it pays them, will find that its wish to be seen to be active about fallout from Chernobyl will lose one of the few markets on which its dependent farmers rely.

The circumstances are curious. The ministry is "erring on the side of caution", as one of its spokesmen said this week, to a degree that may prove economically unwise. Although caesium is one of the predominant radioactive components of fission

products, it differs from iodine, strontium and plutonium in being a generalized constituent of the mammalian torso. The result is that, although the radioactive half-life of ^{137}Cs is roughly 30 years, the residence of the material in people and sheep is much less, more like 30 days than 30 years. The highest concentrations of $^{134,137}\text{Cs}$ have been found in British sheep where rainfall was heaviest early in May (which is where the agriculture ministry looked for them), and in lambs rather than older animals, a factor to some extent offset by the fact that the biological half-life is even less in lambs than in sheep, perhaps as little as 15 days. Yet the ministry has banned the sale of lambs from Britain's soggiest sheep-farms on the strength of observations of levels of contamination in a proportion of the animals sampled which is much less than the $9,000 \text{ Bq kg}^{-1}$ defined as the Emergency Reference Level by the National Radiological Protection Board, an independent public agency. Why the fuss?

Not only domestic politics is involved. Soon after the accident at Chernobyl, the European Commission was stampeded by some of its member governments into an indiscriminating ban on the import of foodstuffs from Eastern Europe. For regulatory purposes, the permissible limit on the radioactive content of food was fixed at 600 Bq kg^{-1} , partly on the grounds that nobody at the outset knew the composition of the fallout, which might have contained more plutonium than appears to have been the case. Now, after the event, the European Commission seems to have been doing its best to justify its decision. A meeting of experts called under the aegis of the Euratom Treaty has apparently recommended that the "action level" for the radioactive content of foodstuffs should be $1,000 \text{ Bq kg}^{-1}$, a factor of nearly ten below that recommended by the radiological protection board. The British ministry seems to have acted on lamb because this limit has been exceeded in some of the animals slaughtered in the past week or so.

Nobody will weep too much for the farmers whose trade will be affected, but the general scare about lamb will have more lasting consequences. It seems to be agreed that the annual dose of radiation arising from an average diet of lamb uniformly contaminated to the $1,000 \text{ Bq kg}^{-1}$ level would be 0.15 mSv , an estimate already biased on the side of caution because the calculation is based on the physiology of a 10-year-old child. This is a small fraction of the annual dose in Britain due to natural radiation, estimated at 2 mSv , and less even than the fluctuation of the natural dose from one part of the country to another. The International Commission on Radiological Protection allows that artificial doses for members of the general population should not exceed 5 mSv in any year, or more than 2 mSv a year chronically. But to acquire even 0.15 mSv a year from this season's British lamb, people would have to start with heavily contaminated lambs, keep them in a freezer and eat nothing else. The agriculture ministry may have done something to protect a few lamb-gluttons from the small risks of their excesses, and at the same time to suggest that it is an action ministry, but the general effect of its hasty decision will be unwelcome.

The particular danger in the ministry's over-active response is that it will reinforce the general opinion that radiation and radioactivity are mysterious and awesome entities. If it should be necessary to ban British lamb from sale several weeks after the passage of a radioactive cloud, who can hope to be safe from it? The truth, of course, is that calculations of the kind on which the ministry based its decision are by no means uncertain. The notion that caesium, radioactive or otherwise, travels through soil into grass and from there into the bodies of grazing animals in a relatively slow fashion is well described. The calculation of the radiation doses that result are intricate but relatively precise. The fact that the doses likely to be received by British lamb-fanciers are so much smaller than the internationally agreed limits and the natural background should help people to live with the knowledge that the damage done by radiation is less certainly calculated. Haste will have the opposite effect. □

British astronomy

Greenwich Observatory to move to Cambridge

THE decision to move Britain's oldest observatory has finally been taken by the Science and Engineering Research Council (SERC). The Royal Greenwich Observatory (RGO) is to move from its present site at Herstmonceux Castle in Sussex to Cambridge, where it will sit alongside the university's Institute of Astronomy.

The decision has come as a relief to many of RGO's astronomers who feared that SERC might try to force through a move to the Royal Observatory at Edinburgh, the course of action recommended in an earlier report and bitterly opposed by RGO staff. Worries over the move have been heightened by the apparent unwillingness of SERC to be frank. The week before last, the matter was raised in the House of Lords and the procedures adopted by SERC for dealing with the case described as "grossly inadequate". In particular SERC failed to make public an earlier report spelling out the options for RGO, and failed to canvass adequately astronomers' opinions.

Opinions are now divided at RGO; some, particularly those among the research-orientated staff, feel there may be new opportunities in a close association with Cambridge. For others a move to Cambridge simply means uprooting homes and family and their official reaction has been "decision received with disbelief". Opposition is certain to continue.

Herstmonceux is RGO's second home, Greenwich having been abandoned in 1948 in search of clearer skies. But now the need for even better viewing conditions means that its activities centre on the new 4.2-m William Herschel telescope being built in collaboration with Spain and the Netherlands at La Palma in the Canary Islands. By 1990, its initial instrumentation will be complete and RGO's 190 staff will probably be reduced to around 100 at the observatory and 30 at La Palma. The move to Cambridge will also take place in 1990.

For SERC, that move is dictated by its corporate plan, adopted in December 1985, to operate its establishments in support of universities and polytechnics rather than as independent research institutes. The thinking behind that is simple enough: with present staff numbers there is time for only a little independent research at institutions, 90 per cent of the effort has to go to developing and managing research facilities for the academic community. Without powerful input from the universities, there is a risk they would

lose touch with the leading edge of research. Thus, the argument runs, it makes sense for RGO to have a close association with other astronomers at a university. Some RGO researchers agree and see a better chance for spending their 10 per cent of research time profitably at Cambridge.

The risk, of course, is that an observatory closely linked to one university will cease to function as an open national institute. Before making the decision to move RGO, the SERC council apparently did consider, and reject, the alternative of building up the observatory so that it could function as a powerful independent institute. Quite how it has arrived at this decision is unclear for the cost of moving the entire institute might seem to be greater than of increasing research there, especially when disruption to the Herschel project at a critical stage in its development is taken into account.

In rolling out the welcome mat at Cambridge, Professor Martin Rees has been at pains to point out that Cambridge will not gain special rights "through propinquity". Some financial savings can be expected as libraries, computers and the like can be shared. The combined force at Cambridge should then be a match for any of the overseas astronomical competitors, like those at Arizona and Pasadena. That in turn should give Britain a foothold in the next generation of giant telescopes where international cooperation is bound to be necessary on an even bigger scale. Even now, a ten-nation plan for a 15–18-m-telescope at La Palma is under consideration.

The loser in the move is the University of Sussex, which, although 20 miles from Herstmonceux, has made considerable use of its facilities and has 7 visiting staff from RGO. The Sussex astronomers — 4 faculty, 6 post-docs and 23 PhD and MSc students — are now very concerned about the future. Although particularly strong in theoretical astronomy, half their work involves observation at RGO. What they will now do has yet to be decided.

One thing not yet clear is how the move to Cambridge is to be paid for. Assurances have been given that the move will be self-financing; that means that the money raised from selling off Herstmonceux Castle will pay for the move. But it is by no means clear that this is really possible; so little information has been released by SERC, and the market for castles is so hard to assess, that the possibility the move will fall through cannot be totally dismissed.

Alun Anderson

Monoclonal antibodies

Kidney rejection at bay

Washington

A MONOCLONAL antibody that reduces kidney transplant rejections last week won approval from the Food and Drug Administration (FDA), marking the first commercialized therapeutic use of the British-born technology in the United States. The drug could benefit over 5,000 transplant patients each year and pave the way for other antibody treatment schemes on the horizon. As a therapeutic tool, monoclonal antibody techniques may, however, involve limitations that have not clouded their success in diagnostics.

The drug, a product of Ortho Pharmaceutical Corporation in Raritan, New Jersey, acts by specifically neutralizing the T cells responsible for renal rejection. In clinical trials, it reversed approximately 94 per cent of rejections in patients receiving antibody intravenously. Conventional steroid treatment ordinarily results in a 75 per cent reversal rate. Because it is targeted to a particular leukocyte, the antibody does not suppress the entire immune system, but it can cause chills, fever and nausea.

Ortho is hoping that its treatment will eventually supplant conventional methods for combating acute rejection. So far, however, the drug cannot be used as a general maintenance immunosuppressive agent (administered over months rather than days) because it is thought that the body may mount an immune response to monoclonals derived from mouse spleen hybridomas. This problem has surfaced before, most notably in cancer treatment, the major focus of monoclonal antibody therapy. Researchers say the only way to circumvent it may be to rely on hybridomas of human cells alone.

Ortho's achievement caps a development drive initiated in 1979, just a few years after the first description of hybridoma culture by Medical Research Council investigators Kohler and Milstein (*Nature* **256**, 495; 1975).

Biotechnology division president Dennis Longstreet estimates a market of between \$15 and \$20 million annually in the United States for the product, which also received approval in France and Switzerland last winter and is currently awaiting word from Italy.

Monoclonal antibodies for cancer treatment are already in clinical trials, and other immunological therapies seem likely candidates for future testing. Despite potential shortcomings, there is good cause to believe that FDA's action ushers in a new era for monoclonal antibody applications.

Karen Wright

US space science

Centaur bites the dust

Washington

SPACE science suffered a further major setback last week with the announcement by the National Aeronautics and Space Administration (NASA) of its decision to abandon development of the Centaur upper stage for use aboard the space shuttle. The decision was taken because safety studies indicated that Centaur, which carries tens of tonnes of liquid hydrogen fuel and liquid oxygen, would constitute an unacceptable risk to the shuttle crew.

The immediate effect of the decision will be to delay until at least December 1989 two major space exploration missions that were to have been launched last month using the shuttle/Centaur launch combination: Ulysses, the European Space Agency mission to explore the poles of the Sun, and Galileo, the NASA/West German mission to explore Jupiter and its moons. The Challenger disaster in January had already ensured that Ulysses and Galileo could not fly before 1988.

Other planned science missions that could have used the shuttle/Centaur combination may also be delayed by last week's decision, including the Magellan mission (formerly known as Venus Radar Mapper), CRAF (Comet Rendezvous and Asteroid Flyby), and Cassini, a Saturn orbiter and probe. But Dr Frank McDonald, NASA's chief scientist, said this week that he was confident that launch problems could be solved in time to avoid delaying these later missions.

According to the Federation of American Scientists, several Department of Defense missions, including the "Milstar" communications satellites, were to have used a version of the Centaur upper stage in the shuttle and will probably also face delays. Centaurs used with expendable rockets such as Titans and Atlases are not affected by last week's decision and may provide an alternative means of launching heavy satellites into Earth orbit or into planetary trajectories.

The shuttle/Centaur combination was already under scrutiny at the time of the Challenger accident, which further increased concern about safety; separate studies have been conducted by NASA and by Congress. In a letter to NASA administrator Dr James Fletcher, Congressmen Bill Green and Edward Boland of the House of Representatives' appropriations subcommittee that deals with NASA wrote last week that "since the accident the yardstick against which... risks will be measured has forever changed".

NASA officials interviewed for Green and Boland's study had mixed views about shuttle/Centaur. Some believed it could be made safe, but it was clear that at least

another \$175–200 million would be needed to do so and to absorb the delays caused by the Challenger accident.

Another new safety worry is that plutonium from the radio isotope thermal generators (RTGs) carried on Ulysses and Galileo could cause 200 cancer deaths and contaminate hundreds of square miles of Florida if it was dispersed in an explosion, according to studies carried out by the Department of Energy. Analyses of the Challenger accident suggest that the overpressures generated in a shuttle explosion are unlikely to rupture the RTGs, but nevertheless special protective shrouds were considered.

The delay to science missions will now depend on how soon the shuttle flies again (not thought likely before December 1987) and on what other launch vehicles can be developed.

Both Ulysses and Galileo were energetically very demanding launches; the Centaur's cryogenic motors represented the most compact and the lightest power source of sufficient capability.

At NASA's Jet Propulsion Laboratory in Pasadena the abandonment of Centaur was no surprise. Staff had been working on alternative launch possibilities for future planetary missions since it became clear after the Challenger accident that the performance of the shuttle would be impaired by the extra weight entailed by safety modifications. An energetically less demanding but more time-consuming delta-vega (ΔV) trajectory was already in prospect for Galileo before last week's decision, and other heavy orbiters of outer planets faced a similar prospect.

According to Allen Wolfe, deputy project manager for Galileo, one possibility for that mission is to use the shuttle with a solid fuel double Inertial Upper Stage. A special third-stage injection module that would be needed has, however, yet to be developed and could not be ready before 1991, according to one estimate. Wolfe had no cost estimates for the re-engineering that would be necessary.

Another possibility would be to use a Centaur with a Titan 34D-7, a new rocket being developed for the Department of Defense. But the new Titans cost more than \$200 million each, and as soon as they become available in 1988 they are likely to be fully booked by the military; current plans call for only five to be built each year between 1989 and 1992. And missions to the outer planets launched from a Titan-Centaur would all have to employ delta-vega trajectories, which can increase total journey time by two or three years. Last but not least, a completely new upper-stage design is by no means ruled out.

Tim Beardsley

Chinese rockets

Stealing a march on the West

LAST autumn, China formally announced that it was willing to launch satellites for other countries on a commercial basis, using its Long-March-2 and Long-March-3 rockets. At that time, this was viewed internationally more as a declaration of Chinese confidence in its space programme than as a serious tender for clients. The Challenger disaster and the Ariane failure last month have, however, forced a number of space agencies to think seriously about the Chinese proposal, particularly now that Western insurance companies are refusing to insure satellites. On 6 June, Mr Sun Jiadong, the Chinese Vice-Minister of Astronautics, announced at a press conference that the People's Insurance Company of China will offer "below-market" insurance rates for such launches.

According to Sun, China has so far been approached by eight potential launch customers; Sweden, the United States, the United Kingdom, the Netherlands, Canada, Australia, Pakistan and Indonesia.

The first country to sign an option agreement was Sweden, for its projected *Mailstar* communications satellite, which weighs only 100 kg and so would in any case be too small for Ariane. But the first country actually to use the Chinese facilities, Sun said, will probably be the United States, as the Western Union Corporation signed a memorandum with China last month on the launching of two communications satellites.

At present, China has the capacity to launch 10–12 rockets per year, Sun said. The maximum payload is 1.4 tonnes, but this will shortly be raised to 2.5 tonnes. An obstacle to the conclusion of launch contracts was, according to a US space team which visited China last year, that the Chinese do not provide good ground test data on their vehicles. But what appeared a major obstacle in 1985 may well not seem so in the aftermath of Challenger and Ariane.

Foreign clients will not be able to be present at the launch site at least for the time being. But this, according to Mr Shen Rongjun, vice chairman of the Science and Technology Committee of the Commission of Science, Technology and Industry for National Defence, is simply because there are no facilities for guests at the site. Foreign visitors are welcome, he said, at the Jiuquan centre, which has received more than 30 such delegations in the past few years. As soon as the necessary conditions are provided at the launch site, Shen said, visitors will be welcome there too.

Vera Rich

Environment

Strategy for Earth systems

Washington

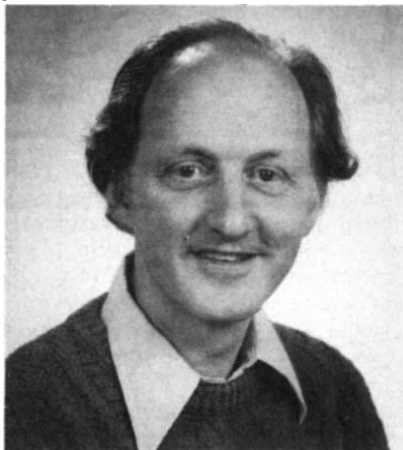
ENTHUSIASM is running high for a new report released today that charts a course for government-sponsored efforts to understand global environmental changes in the coming decades. Written by the Earth System Science Committee (ESSC) for the National Aeronautics and Space Administration (NASA) and the National Oceanic and Atmospheric Administration (NOAA), the report lists specific roles for NASA, NOAA and the National Science Foundation (NSF) in reaching a better understanding of how Earth systems interact on a global scale. But even supporters of the report's goals acknowledge that finding the money and political will to achieve its objectives will not be easy.

Francis Bretherton, former director of the National Center for Atmospheric Research in Boulder, Colorado, and now a senior scientist there, was chairman of ESSC. Bretherton says the report was an attempt to provide a strategy for what must be done to look at the Earth as a system. By understanding these interactions, says Bretherton, it will be possible to predict changes that will occur from both natural processes and human activities over the next century.

NASA originally sought the report to provide priorities in its Earth science programmes, in much the same way that previous reports provided direction in astronomy and planetary science. But to achieve an integrated picture of the Earth, it was clear that other agencies would have to become involved, and NOAA administrator Anthony Calio requested that the report be directed to his agency as well.

The report divides the needs of Earth sciences into two periods; the years preceding the launch of the US manned space

station, and the decades that follow. In the pre-space station era, a coordinated sequence of missions is proposed for achieving the goals of Earth system science. Some, such as the Upper Atmosphere Research Satellite and the Navy's



Francis Bretherton

Remote Ocean Sensing Satellite, have already received budgetary approval. Others, like the Ocean Topography Experiment (TOPEX) included in NASA's 1987 budget request and the Geopotential Research Mission, proposed as a new start in 1989, still await approval. The ESSC endorsement should have a positive effect on their chances of receiving support.

In the post-space station era, NASA's emphasis will shift to the Earth Observing System (EOS), a series of instruments designed to fly either aboard the space station itself or on polar orbiting platforms serviced by the space station.

The ESSC report calls for greater coordination in the activities of NASA, NOAA and NSF. It suggests the immediate adoption of an advisory body to

guide management choices among the agencies. Especially important in the coordination process is the establishment of information storage and retrieval processes for handling the avalanche of data generated by space-based Earth-observing missions as well as *in situ* measurements.

NOAA has traditionally been responsible for gathering and storing such data. But NOAA has not been a favoured agency in the Reagan administration, and many, including Bretherton, have expressed doubts that NOAA's budget will allow it to keep up with the data demands. Administrator Calio is enthusiastic about the goals of the Bretherton report, but also wonders where the money will come from.

Some activities are already under way at different agencies that will dovetail with the recommendations of the ESSC report.

NOAA is developing NOAANET and NOAAPORT, systems for disseminating operational weather satellite data to climate researchers. NOAA is also the lead agency for the Tropical Ocean Global Atmosphere Program (TOGA) designed to gain a better understanding of the El Niño phenomenon. But the administration's proposed budget reduces NOAA's support of TOGA by approximately half in the 1987 budget.

NSF has begun the Global Geosciences programme, seven programmes including the World Ocean Circulation Experiment and the Global Atmospheric Research Program, that represent a major attempt to realize the priorities set by the National Research Council's International Geosphere/Biosphere Program.

For NASA, the Bretherton report comes at a time when the agency is facing many questions about its future. Both the Challenger accident and the decision to abandon the Centaur upper stage rocket for shuttle-launched missions (see p.800) will have a profound effect on NASA's scientific activities.

NASA administrator James Fletcher is well briefed on the concerns of Earth system scientists, but may be unable to spare agency resources to achieve their goals while trying to return the shuttle programme to operational status.

D. James Baker, a member of ESSC and president of the Joint Oceanographic Institutions, argues that the report correctly identifies the good match between emerging technologies and the scientific needs of Earth science. Baker sees the report as non-controversial, scientifically sound, and likely to receive support in Congress. But NASA's internal turmoil and the lack of a strong champion inside the White House may lessen the report's impact. The ESSC report tried to "strike a balance between program needs... and a realistic demand on agency resources and capabilities." The question is, did it succeed?

Joseph Palca

Turkey turns away Israeli scientists

Rehovot

TURKEY is pleased to have Israeli tourists, but seems to discourage visits by Israeli scientists.

This is the conclusion drawn by Professor Mordechai Magaritz of the Weizmann Institute and three of his professional colleagues from the Israel Geological Survey, all of whom have failed to obtain visas in order to attend an International Union of Geological Sciences Workshop on Late Permian and Triassic in Western Turkey which begins in Istanbul on 14 July.

They sent their \$250 registration fee to Turkish scientist Remzi Akkok on 11 April and then, several weeks ago, went to the Turkish Consulate in Tel Aviv to obtain a visa. There was a long line of Israelis

already waiting, all of whom obtained visas.

Only Magaritz and his friends were turned down because, the consul explained apologetically, they were scientists, not tourists. Scientists require special permission from the Turkish Foreign Ministry, which has not so far been forthcoming.

As soon as he made his first, unsuccessful application for a visa, Magaritz asked a Swiss member of the organizing committee, Dr Aymon Baud, to intervene. Baud, Magaritz's co-author on a paper that Magaritz had intended to present at the workshop, immediately contacted Akkok, but to no avail. Magaritz has now turned to other members of the organizing committee in the hope that they will be able to influence the Turks. Nechemia Meyers

US defence research

Few second thoughts at MIT

Boston

HALF of the faculty polled at the Massachusetts Institute of Technology (MIT) claim they would discontinue their research if their areas of research became militarily sensitive. Such aversion, however, has not lessened MIT's appetite for contract work from the US Department of Defense (DoD). In a DoD report on university contractors, MIT was identified as the largest on-campus university contractor, a position it has held for five of the past nine years.

Concern over the scale of this involvement has led to the setting up of a faculty/student committee to gather facts about the military presence at MIT. The data gathered, based on student and faculty questionnaires as well as university records, were presented at the faculty meeting last month.

Although DoD funds are expected to increase by 20 per cent from fiscal year 1985 to fiscal year 1987, the committee claims that defence is not the most important source of research backing at MIT. DoD support, in constant 1985 dollar volume and total share, is less than it was twenty years ago. Nor is DoD, with 16 per cent, the primary or even the largest on-campus sponsor: in 1985, support was also provided by the National Science Foundation (NSF, 14 per cent), industry (14 per cent), the National Aeronautics and Space Administration (NASA, 5 per cent) and the Department of Health and Human Services (17 per cent). Very few faculty members rely on just one source.

The Department of Energy (DoE), with 23 per cent, has been the largest source of research support at MIT since 1978. Major recipients of DoE support are the Laboratory for Nuclear Science, the Plasma Fusion Center and the National Magnet Laboratory. DoE, DoD and NASA sponsor nearly half of MIT's research, but only 10–11 per cent of that support is for "specific military application".

Off-campus laboratories involved in military research complicate comparisons between universities as well as judgement about the effect of such research on a given university. Data about these laboratories are inconsistently included in university figures, and defence support of such a laboratory has a variable effect on campus environment. For example, MIT's Lincoln Laboratory, with which Paul Gray, president of MIT, would like to see closer campus interaction, is 97 per cent supported by DoD.

The degree of financial support from different federal agencies has fluctuated by presidential and congressional whim.

Thus according to Gray, President Reagan's reversal of President Carter's alternative energy policies has reduced DoE support at MIT. For the most part, however, Gray characterizes federal agency funding at MIT as "stable".

This cannot be said of support from industry, although nobody is downcast by that. Industry has trebled its support of MIT research in the past few years. Gray welcomes that increase, ascribing it to a concerted effort by the university.

Only 5 per cent of the faculty polled think involvement in the Strategic Defense Initiative (SDI) will be good for MIT, with 40 per cent characterizing it as "generally bad". MIT is a top SDI contractor, with SDI providing 25 per cent of Lincoln Laboratory's \$274 million budget in fiscal year 1985. But Gray thinks that \$58 million skews MIT's research image: he points out that the percentage of on-campus research supported by SDI, \$1 million out of \$280 million, is very small. And even if all proposals were funded for next year, that amount will not rise above \$5 million, says Gray.

Many faculty members, according to Gray, feel it makes sense to solve the basic and applied sciences issues of SDI regard-

less of the system's usefulness. Because SDI is as mission-oriented as DoE, however, says Gray, its research support is just as capricious. The fear of an abrupt loss of funding, if SDI is bargained away or declared unfeasible, chills researchers' willingness to rely on the programme.

SDI and military involvement in general evoke a less negative response in the School of Engineering than in other departments. Engineering has a disproportionate share of DoD funding as well as support from major defence contractors. More of that faculty would accept a requirement for security clearances as well as other limitations, and fewer of its students are averse to military work.

Seventy-five per cent of the faculty polled thought that concentration on SDI would channel research away from the civilian economy. This concern was also voiced by Gray in a telephone interview. Basic research garners only 10 per cent of total federal agency funding of the universities. Overall increases in DoD spending can easily mask decreases in that tiny fraction says Gray, resulting in an imbalance harmful to the growth of US science.

At its May meeting, the faculty empowered Gray to appoint a new committee to study the university policy implications of the data reported. The new study will require at least an academic year.

Elizabeth Collins

A prize for Tooze from EMBO

A NEW prize has been launched by the European Molecular Biology Organisation (EMBO). A medal and DM15,000 (£4,500) is to be awarded each year to an outstanding young European molecular biologist:



the aim is to try to help European scientists to gain international recognition as quickly as their US counterparts.

The announcement of the first medal winner at the recent EMBO symposium caused something of a stir: it went not to a research worker but to EMBO's own executive secretary, John Tooze, who from

now on will be administering the prize. A somewhat embarrassed Dr Tooze modestly described the decision as "just an interim measure", with the medal to go to young research workers in the future. But there are many scientists who are happy to admit that Tooze's name is one of the best known in European molecular biology.

Tooze joined EMBO in 1973, after a few years as deputy editor of *Nature* and research administrator at the Imperial Cancer Research Fund. In the award ceremony he was particularly thanked for having built up EMBO's fellowship programmes. EMBO now offers 160 long-term fellowships each year, largely within Europe, and 250 short-term fellowships, as well as running 20 practical courses and 20 workshops and symposia. Then there is the *EMBO Journal*, set up by Tooze in 1981, and with all of the 1,200 or so papers submitted each year passing through his hands.

EMBO's total budget is now 4.8 million ECU (European Currency Units, £7.2 million) and the medal, for which funds were raised from private industry, will help to bring EMBO the publicity needed for further expansion. But its chief aim will be to give young molecular biologists the early recognition that will boost their careers.

Alun Anderson

Soviet science

Too many at the top

THE Soviet Union's "sectoral" research establishments — the institutes, laboratories and design centres that belong neither to the universities nor to the Academy network — have failed to respond to Mr Gorbachev's call for streamlining and restructuring. Reporting to the Supreme Soviet last week, Nikolai Ryzhkov, chairman of the USSR Council of Ministers, noted that some of these institutes had virtually ceased to do science altogether and had become little more than "managerial adjuncts" of the ministries to which they are attached.

During the past 20 years, the network of sectoral institutes has proliferated, as Mr Gorbachev pointed out during the General Committee plenary meeting in June 1985. As a rule, interministerial bureaucratic barriers made the sharing of facilities impractical or impossible, while some establishments seem to owe their origin to the empire-building propensities of certain ministries. Mr Gorbachev therefore called for the closure of redundant, inefficient or outdated sectoral institutes, and the establishment of inter-sectoral scientific and technical complexes that would cut across ministerial barriers and, at the same time, link research facilities with production enterprises. At the same time, a massive injection of finance into science was promised. Annual expenditure (excluding capital outlay) is to rise from 24,800 million rubles in 1985 to 33,000 million rubles in 1990. Capital investment in science will rise by 70 per cent over the same period.

The ministries, however, failed to respond to the challenge. Instead, they leapt to the defence of the institutes threatened by Mr Gorbachev's attack, even if, for years, they had shown no interest in how these institutes were working. In the end, Ryzhkov told the Supreme Soviet, the Council of Ministers had to intervene and close down some of the most blatantly inefficient establishments. He cited two such cases. The All-Union Research and Design Institute for Complex Technological Processes, which belonged to the Ministry of Chemical and Petroleum Machine Building consisted, at the time of its closure, of 71 "ordinary personnel" and no less than 176 "leaders of different ranks", while out of its 130 research projects, only one or two had any scientific relevance. Likewise, one of the design institutes of the Ministry of the Machine Tool and Tool-Making Industry, which employed 600 people at the time of its closure, had managed to produce inventions from only two of its projects.

Vera Rich

Telecommunications

Japanese cable competition

Tokyo

INTERNATIONAL telecommunications in Japan are booming. Annual growth in volume is 20–30 per cent, and in the past few weeks two giant consortia have emerged that hope to grab a slice of this market, currently worth more than 200,000 million yen (£800 million) a year but monopolized until now by Kokusai Den Shin Denwa (KDD). KDD, in turn, has announced plans to lay two submarine optical fibre cables to provide high-capacity digital links between Japan and the American and Asian continents by 1990.

Japan's telecommunications laws were liberalized more than a year ago. Five domestic telecommunications companies have been set up to compete with Nippon Telegraph and Telephone Corporation (NTT) in the domestic market, but in the past month a spate of announcements have heralded competition in the international arena.

First off the mark were the three former *zaibatsu* trading houses, Mitsubishi Corporation, Mitsui & Co. and Sumitomo Corporation, which will set up a feasibility study company next month as a first step towards forming their so-called "second KDD". The consortium will lease transponders of Intelsat satellites from KDD, Japan's Intelsat representative.

Next, C. Itoh & Co., a trading house rival of the *zaibatsu* group, announced plans to establish a new international telecommunications company jointly with Toyota, General Motors of the United States and Britain's Cable & Wireless. This consortium intends to lay its own submarine trans-Pacific optical fibre cable in direct competition with that of KDD.

Both consortia have capital investment links with Japan Communication Satellite, one of the five new domestic telecommunications companies, and so will have ready access to a domestic carrier.

The KDD submarine cable network will be built in collaboration with American Telephone and Telegraph, Korea Telecommunication Authority and, surprisingly, Cable & Wireless (Hong Kong) Ltd. Two branching cables will be laid.

The first, trans-Pacific cable 3, to be completed by late 1988, will link Japan, Guam, Hawaii and the US mainland via a submarine branching point about 1,500 km north of Guam. More than 13,000 km of cable will be laid along the seafloor which plunges to depths of over 8000 m in the trenches that rim the western Pacific in an arc between Guam and Japan. The second cable will run off the Pacific coast and split north of Okinawa, with one branch running to South Korea, the other to Hong Kong.

The total cost of the two cables will be a

staggering \$800 million (£530 million), but with 7,560 channels, the trans-Pacific 3 cable will have about ten times the capacity of the two existing coaxial cables and will be on a par with Intelsat satellites which currently have 6,000 or 12,000 telephone channels. Digitization will allow high-speed data transmission, video transmission and teleconferencing — capabilities that were formerly the preserve of satellites. At present about 60 per cent of KDD's international transmissions are via Intelsat satellites, but this percentage is expected to drop substantially.

Once in place, maintenance costs for the cables should be low, provided submarine slides, fishing trawls or unwary research vessels do not break them — not uncommon events off Japan's coast. For example, in 1972 turbidity current severed the trans-Pacific cable in Sagami Bay and in 1984 the *Natsushima*, mother ship of Japan's deep-sea submersible *Shinkai 2000*, managed to do the same thing at a cost to KDD of ¥52 million (negotiations for compensation are proceeding), while the Japan–China submarine cable has been fished up so often by trawlers it has had to be buried 70 cm below the seafloor using a special cable-laying device equipped with a plough. But barring such accidents, the submarine cable can be expected to have a lifetime of at least 20 years.

The plan of the C. Itoh consortium to lay a competing trans-Pacific optical fibre cable is meeting resistance. KDD considers it to be "unfair competition" because, as a public entity, KDD has a responsibility to maintain parallel systems (satellite and cables) to ensure continuity of service if one system broke down, whereas the "third KDD" plans to use only cable.

KDD further argues that there is simply not enough room for three competitors in Japan's international telecommunications market. Critical in this argument is the future size of the market. Whereas KDD estimates it will amount to 600,000 million yen by 1995, a survey released by the Federation of Economic Organizations (Keidanren) thinks it will be twice that. Competition will influence market value. If rates are slashed, market value growth will not match the growth in telecommunications volume.

The fate of the third KDD thus remains uncertain. Toyota has made no clear commitment to join the consortium, and officials at the Ministry of Post and Telecommunications echo KDD's view that "two's company, three's a crowd". The consortium, however, may simply have been floating a trial balloon.

David Swinbanks

Scientific fraud

New Public Health service policy

Washington

AFTER a delay of four years, the US Public Health Service (PHS) has inaugurated a formal policy for dealing with allegations of scientific misconduct such as fabrication and plagiarism. Scientific staff and officials of organizations receiving PHS funds must in future report alleged misconduct to designated institutional "misconduct policy officers". The new policy also requires institutions that receive its funds to certify that they have in place procedures for investigating allegations of misconduct, and to inform the funding agency when formal investigations are initiated.

The need for a formal PHS policy became apparent after a spate of widely publicized cases of fraud, plagiarism and other misconduct in biomedical research in the early 1980s, of which John Darsee's fabrication of research results was the most infamous. The effort to develop ways of dealing with allegations effectively while minimizing damage to the reputations of those unjustly accused was instigated in 1982. Congress instructed PHS to develop a policy last year.

The new policy has been developed chiefly by the Office of Extramural Research and Training of the National Institutes of Health (NIH), which has in recent years overseen NIH misconduct investigations on an *ad hoc* basis. The deputy director of NIH for extramural research and training, currently Dr William Raub, is "misconduct policy officer" for the whole of PHS. Fifty allegations were received by Raub's office in 1985, of which twenty-four proved substantial; the frequency of allegations has increased markedly since the early 1980s, for reasons not clearly understood.

The policy defines misconduct as either a "serious deviation" from accepted research or reporting practices, or material failure to comply with requirements such as those protecting human subjects or animals. Institutions are required to conduct thorough reviews of any allegation that is "not frivolous or unsubstantiated", preferably within 60 days, and to keep NIH informed about the investigation.

Suggested sanctions range from a simple reprimand to imposition of special reporting conditions and debarment of individuals or institutions. Criminal misconduct such as misappropriation of funds is excluded from the policy and dealt with by separate procedures.

The policy now implemented by PHS is likely to serve as a model for similar policies in other research agencies; the National Science Foundation, for one, expects to implement an almost identical policy. The Office of Management and

Budget has recently proposed requiring all federal agencies that award research grants to develop policies for debarment, necessary if debarment is to withstand a court challenge.

The detailed PHS policy guidelines, which include sections for agency personnel and for institutions that receive grants, outline procedures for conducting investigations and for determining administrative action. Individuals under investigation must always be so informed; otherwise, the fact that an investigation is in progress is restricted to those who need to know. As far as possible, informants' identities are kept secret.

Although the announcement of the PHS policy appears to have been somewhat muffled, it was approved by acting assistant secretary for health Donald MacDonald in April and came into force immediately. The policy retains "interim" status until incorporated into standard guidance documents for agency staff.

The interim policy would establish a PHS-wide "alert" system to notify awarding institutions when grant applicants are under investigation for misconduct or are subject to restrictions resulting from adjudged misconduct. Individuals flagged in the "alert" system are not necessarily precluded from receiving an award or participating in review committees; decisions are made according to circumstances.

Although NIH themselves already have an "alert" system, its extension to the whole of the Public Health Service will under the Privacy Act require publication in the *Federal Register*, as it entails maintaining a database referencing individuals.

Details of the responsibilities of institutions receiving funds have also yet to be published for comment before the policy can be put into force; both *Federal Register* notices should be published in August.

The new policy does not answer all the demands of those who have been critical of PHS's existing informal procedures. Institutions are required to inform their awarding agency of an investigation only if preliminary inquiries suggest that the allegations are serious and plausible; some contend that perceived pressure on an institution not to find evidence of wrongdoing will jeopardize impartial scrutiny. But NIH did not want the responsibility of investigating every allegation made, and after long debate decided that primary responsibility should rest with the host institution. NIH can follow up with their own investigation if the institution's appears inadequate.

Daryl Chubin of Georgia Institute of Technology, who has studied scientific misconduct for the National Science

Foundation, comments that the existence of guidelines should make it easier for those who suspect misconduct to make their allegations confidentially. But Chubin still sees conflicting pressures on institutions that have to investigate fraud; as employers, they should normally be prepared to give employees the benefit of the doubt.

Ned Feder and Walter Stewart of NIH, in an unpublished manuscript given in testimony to Congress, have supported random audits of all published research. Such random audits are conducted by the Food and Drug Administration to ensure the veracity of data it uses in making regulations, and the agency can impose sanctions on an investigator who is found to be doing careless research. PHS's policy is, however, constrained to scrutinize only the more serious allegations.

Tim Beardsley

JET Britons seek to

BRITISH fusion physicists working at the Joint European Torus (JET) near Oxford take home less than half the pay of other European nationals on the JET project, even though they have equivalent jobs. This is blatant discrimination, the European Court of Justice heard in Luxembourg last week, two-and-a-half years after the British JET staff first instructed European lawyers to take up their case.

Last December, the Court's Advocate General recommended that a subsidiary court accept the Britons' arguments, and award them back pay. However, the subsidiary court referred the case onward to full court, which heard the oral evidence last Thursday. According to the European Commission, the back pay if awarded would amount to some 10–15 million ECU (European Currency Units: roughly 1 ECU=\$1, or £0.67). Distributed among the 222 British JET staff involved, this would amount to an average windfall of 45–65,000 ECU (£30–45,000) each, and a salary henceforth of twice present earnings. The Commission estimates that JET costs would rise by 2 million ECU annually, around 2 per cent of current expenditure. This would give the British an average pay rise of £9,000.

Perhaps unsurprisingly, 75 per cent of the Britons working at JET have contributed to a legal fund to pay for the case in Luxembourg, which is based on the argument that the JET statutes discriminate against British employees and so contravene the Treaty of Rome the basic legal text of the European Economic Community (EEC). EEC pays for JET through its Euratom component. The Treaty of Rome demands equal treatment of all EEC nationals, but the JET statutes, agreed (perhaps illegally, it now seems) by the European Council of Ministers in 1978,

US biological weapons

New test facility defended

Washington

DESPITE delays in construction forced on it by the courts, the United States Army has defended its plans to build a biological aerosol test facility (BATF) at the Dugway proving ground. In a report to Congress, the Army calls BATF a "small but critical facility" needed to provide adequate defence against biological weapons. But critics worry that the facility may easily be mistaken for the harbinger of a new offensive biological weapons programme, putting arms control agreements at risk.

Language inserted into last year's defence appropriations bill required the Army to respond to a set of specific questions about the planned uses of the Dug-

way facility. In its written response, delivered to Congress earlier this month, the Army states that "the United States cannot defend itself adequately against conventional biological warfare agents", making increased defensive efforts crucial. Two factors convince the Army that greater vigilance to the threat of biological warfare is necessary. The United States still maintains that yellow rain in South-East Asia is evidence of Soviet use of biological weapons in violation of the 1972 biological weapons convention.

In addition, new biotechnologies provide cheap and easy methods for production of biological agents that can be used as weapons. BATF is designed to test defensive equipment against a variety of potential biological agents. Despite the fact that Army plans call for a biosafety level 4 facility, the level traditionally associated with recombinant DNA experiments involving virulent agents, the Army has no plans to test genetically engineered material in BATF.

But critics feel the Army's own arguments will force it to resort to testing recombinant DNA organisms. Barbara Rosenberg, a molecular biologist at the Sloan Kettering Institute in Rye, New York, and a member of the committee on the military use of biological research, argues that developing a defence against the many agents formed by recombinant DNA techniques will require similar technology. Indeed, the Army report does not permanently rule out such activities.

The Biological Weapons Convention prohibits signatories from developing, producing or maintaining stockpiles of biological weapons. The Army maintains that BATF does not violate the convention because research on biological agents for "prophylactic, protective or other peaceful purposes" is permitted by the treaty. But critics point out that toxic agents used for testing could quickly be transformed into offensive stockpiles. Possessing such a capability only heightens uncertainty about US intentions, says Rosenberg.

The Dugway facility has faced problems since it was proposed in 1984. At first, the project was held up by Senator Jim Sasser (see *Nature* 312, 189; 1984). After Sasser's objections were resolved, Jeremy Rifkin successfully thwarted Army plans by convincing a federal judge that the Army had not made an adequate environmental assessment for its facility. The Army has now commissioned a complete environmental impact statement due to be completed at the end of this year. But even an acceptable impact statement may not persuade the court to lift its injunction blocking construction of BATF. **Joseph Palca**

Chernobyl

Eastern bloc reactions

THE chief inspector of the Czechoslovak Atomic Commission, Jiri Beranek, has called for a re-evaluation of all nuclear safety measures, on both a national and an international level. Interviewed on Prague radio, Beranek stressed the need for a longterm study of possible chemical reactions within reactors that would lead to the generation of hydrogen, the presence of combustible material in reactors, and the reliability of the human factor.

The first two points clearly relate to the Chernobyl disaster. Although the Soviet government's special commission on Chernobyl has not yet reported, Soviet experts have already accepted a scenario in which a surge of power led to the production of steam, which reacted with the zirconium fuel-cans to produce hydrogen. Whether human error was directly involved at Chernobyl is unclear. In mid-May, the Soviet media made brief mention of an "experiment" being in progress at the time of the accident, which could mean that someone tried some unorthodox method of dealing with the reactor which was already causing problems.

Beranek's concern for increased nuclear safety coupled with a firm commitment to nuclear power is typical of Comecon officials — although most of them, privately, have a good store of "black" post-Chernobyl jokes. In Poland, where some 300 inhabitants of the Bialystok area signed a petition calling for a halt to construction of the country's first nuclear plant at Zarnowiec, the official reply was that a future without nuclear power would put Poland back in the dark ages.

In Hungary even the dissident ecological "Danube Circle" has refused to condemn nuclear power, which it sees as less threatening than the controversial Gabeikovo-Nagymaros hydroelectric dam. And the Soviet Union itself, of course, is still committed to an energy policy based on the "accelerated growth of nuclear power", which, according to the current five-year plan, should produce an output of 390,000 GW in 1990 (against 167,000 GW in 1985).

It is only the Soviet Union's non-aligned and neutral neighbours that changed their energy strategy in the aftermath of Chernobyl. Yugoslavia, which already had a vocal antinuclear lobby, has postponed any decision on further nuclear power plants "until the long-term programme for the development of the energy industry has been adopted", and has decided to concentrate on energy conservation and hydroelectric power. And Finland has likewise dropped plans for a fifth nuclear power plant. **Vera Rich**

double their pay

have Britons employed by the United Kingdom Atomic Energy Authority on British salaries, while non-Britons (some 162 in all) are employed by Euratom on the very comfortable salaries of a European civil servant.

The European Commission is resisting any change in the JET salary arrangements, however, and an official pointed out on Monday that there were precedents. Euratom helps support a number of other European fusion research centres besides JET, though these are national (such as Britain's own laboratory at Culham, France's at Fontenay aux Roses, and West Germany's at Garching). There are a number of non-nationals (30 at Fontenay, 10 at Garching, but just one at Culham) who are supported at these institutions on Euratom contracts somewhat more favourable than the local, national pay scales. JET is unique, however, in having a roughly 50:50 ratio of local and Euratom-supported staff.

According to a spokesman for the British group at JET, the loyalty and commitment of the Britons to the JET project remains high, and some of the Euratom employees at the laboratory are supporting the case. The late director of the JET project, Hans-Otto Wüster, in fact first suggested the Britons seek legal advice, although he resisted that advice when it proved favourable to the Britons' case. Morale is good at JET, staff insist, and the case is being pursued "without passion". The European Commission hopes for a court decision by the end of this year. Meanwhile, the British at JET are waiting for the recommendations of the new Advocate General appointed to the case; unfortunately for them, the first, who found in their favour in the lower court, has now retired.

Robert Walgate

Future of lion tamarins

SIR—On behalf of the International Recovery and Management Committee for the golden-headed lion tamarin, I was delighted to read the News item entitled "Tamarins to go home" (*Nature* 320, 674; 1986). Tom Milliken, director of WWF Traffic (Japan), is to be complimented on his contribution to the lobbying and diplomacy that has culminated in the announcement that the ten golden-headed lion tamarins imported illegally into Japan three years ago will be returned to the ownership of the Brazilian government. Conservationists well recognize that the case holds great significance for the future development and implementation of CITES in Japan.

With regard to the reference in the news item that captive breeding programmes are under way at the Rio de Janeiro Primate Centre and at the zoo in Jersey, however, Jersey does not at present have this species included in its successful breeding programmes for golden lion tamarins.

Dr Ademar Coimbra-Filho, director of the Rio de Janeiro Primate Centre, and I are co-chairmen of the international committee. At the inaugural meeting of the committee at San Diego in June 1985, the main objectives were agreed to be illegally exported or illegally held specimens confiscated or voluntarily replaced under the trusteeship of the Brazilian government and to use the world's best resources to establish a viable captive breeding population under scientific management. The committee advises the Instituto Brasileiro de Desenvolvimento Florestal (IBDF) on the management of the species in captivity.

The release into the wild of these already displaced animals is not recommended at this time; the information available on population, distribution and habitat protection in the Brazilian state of Bahia is insufficient to guarantee survival and could well, through disease, impair the survival of the remnant population.

The recent successful re-introduction of the closely related golden lion tamarin species into the Poco das Antas Biological Reserve in the State of Rio de Janeiro required many years of careful preparation. Similar preparatory research must be carried out before such re-introduction is undertaken with the golden-headed lion tamarin and, in the meantime, a captive self-sustaining population must be secured, with breeding programmes being established at a number of different locations, but managed as an overall single unit.

As a result of a recent ballot among the International Management Committee, of which 50 per cent comprises some of Brazil's leading conservationists, it was unanimously agreed that individuals of the golden-headed lion tamarin species should be made available only to institu-

tions that have already had experience with the long-term maintenance and successful propagation of the golden lion tamarin. Priority has been given to selected captive breeding institutions in Brazil, and a list of suitable locations outside Brazil has been drawn up in order to receive individuals that the Brazilian Conservation Authorities consider are surplus to their requirements.

We have great hopes. The golden lion tamarin programme has nurtured a captive population from approximately 70 specimens in 1972 to more than 400 individuals by 1985, recently recording an annual population growth of 50–60 animals, on which was based the successful re-introduction programme for this species.

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Is this science?

SIR—In the past few years, we have witnessed in the pages of *Nature* a curious debate between those broadly described as "evolutionist" and "creationist", adopting, the former would have us believe, scientific and unscientific arguments respectively. Leaving aside the deterioration into personal abuse, reminiscent more of discussions in a school playground than reasoned debate in a learned journal, it is particularly disturbing to find those purporting to defend the scientific process haplessly resorting to the same sort of emotional and illogical tirades assumed to be the hallmark of the opposition.

In the latest example, J. Richard Wakefield (*Nature* 320, 392; 1986) asserts that, in contrast to those who believe in a created order, science "accepts nothing on faith". This naivety requires no other comment than to point to a prime example of the author's own article of faith, that "nothing is potentially beyond science". This happens to be a viewpoint that I share. But let us recognize the ill-defined nature of the belief.

Selective awareness is evident in both the statement that "misrepresentation runs rampant in creationism" and the list of human failings in the last paragraph. Is it possible he really believes that no scientist has ever fallen into this category? Few would argue that scientists are perfect. For those who do, a glimpse at the history of palaeontology and the recent debate about cladistic thought at the British Museum (Natural History) ought to convince them otherwise, so "pure" science is probably rather thin on the ground. It seems to me that if colleagues feel the need to defend science against some of the more

bizarre claims levelled at it, they must avoid dual standards. It is our responsibility to counter false logic with rigorous argument, not with personal invective or dubious claims to infallibility.

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Last word on metric

SIR—My passing remarks mildly critical of metric units (*Nature* 316, 480; 1985) have provoked your correspondents (317, 10; 1985; 318, 203, 596; 1985; 319, 8, 93, 716; 1986) to imply that I am in league with those who have opposed printing, telescopes, railways, telephones, motion pictures and so on. That is not quite accurate.

While metric units certainly have some role in science and technology, little purpose is served by compelling their everyday use. One might as well argue that since English is now the lingua franca of science, its use should be made compulsory in households the world over. While that might be a fine idea on its own merits, it would likewise be a *non sequitur*.

Why not simply let people use the units they prefer? Imposing alien units merely fuels the common belief that technocrats seek to control all aspects of life. Such imposition also severs society from its rich linguistic and cultural heritage. It will be remembered that metric units were designed by a group of zealots who hoped to do precisely that, their target being the *ancien régime*.

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This correspondence is now closed.
Editor, *Nature*.

Sex discrimination

SIR—Joseph Palca's report (*Nature* 321, 371; 1986) on the possibility of sequencing the human genome raises the problem of which individual's DNA to use, and suggests possible ways of choosing "him" or "her". While I appreciate this egalitarian phraseology, it is not possible to sequence the whole genome using a female source. Some 2.5×10^7 base pairs of considerable importance to half the world's population would be missing.

Perhaps this conflict between scientific and social requirements could be resolved by selecting an XY female as the source of the DNA.

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New technology of medicine

The transformation of basic medical science has been a surprise to all of us, but especially to physicians.

LAST week's *Nature* conference in London was originally planned as one of a series. The intention was that the journal should continue in this vein with its traditional function of acting as a midwife between specialized laboratories and the wider audience outside. Experience has shown that there is a particular need of means by which people who used to be in academic life, but who are now elsewhere, in industry perhaps, may renew acquaintance at first hand with research. Most people were last week surprised that, no doubt more by luck than judgement, a meeting on the biotechnology of medicine appeared to coincide with the fruition of several threads of research in the field.

In retrospect, there is a surprise to be explained. In 1975, when the air was full of reports by government committees (and others) on the promise of biotechnology, the most common prediction was that the pharmaceutical industry would be quickly overwhelmed by a torrent of new products, artificially engineered versions of naturally occurring materials. And, to be sure, human insulin was quickly on the market, although not at such a price that it could hold off competition from chemically modified pig insulin. Since then have come human growth hormone, with an invaluable role to play in the treatment of disease, and the various interferons whose value has still to be defined. There remains a small army of biological materials still waiting in the wings, and there is no reason why the prospectuses of a decade ago will be found to have been bogus. But, inevitably, there is some disappointment in the air.

Much the same is true in the genetic manipulation of plants. Ten years ago, people talked endlessly of the transformation of agriculture that would come about when it proved possible to transplant nitrogen-fixing genes from leguminous plants to those that have evolved without them. There were even academic arguments on the esoteric question whether the effort would be worthwhile in temperate latitudes, where the intensity of sunlight is not enough to make full use of nitrogen-fixing genes. In reality, as events have shown, the nitrogen-fixing genes are a complex too large and well-tuned to be shifted easily from one plant to another. A better hope is that plants at present unable to fix nitrogen directly might, by engineering, be given the facility to form symbiotic associations with soil bacteria (see Jensen,

J.S., *et al. Nature* **321**, 669; 1986). But that, too, will not happen the day after tomorrow, especially if modest agricultural experiments with engineered bacteria are held up for regulatory reasons.

None of this implies that last decade's promises on behalf of biotechnology were false. Did it not, after all, take more than a century to bring the steam engine from a dream to a reality? It is no shame that biotechnology has been required to mature. But the surprise is that so much has happened in medicine, in the treatment of patients, when these techniques were overshadowed by the hopes for new pharmaceuticals.

That monoclonal antibodies would be important has, of course been clear since the beginning, just eleven years ago. The principle that it is possible to make by this means a sensitive reagent for almost any antibody is well established now. But did you know that the more distant hope of coupling monoclonal antibodies to toxins so as to kill unwanted cells specifically is already in the early stages of clinical testing? That is what Professor Robert Baldwin (University of Nottingham) explained last week; among the unexpected findings so far is the discovery that the immune system can make antibodies against toxins when these are coupled to antibodies, which is a hurdle to be overcome. The notion that monoclonal antibodies can be used to sterilize the mouth and teeth against infection by streptococci (Professor Thomas Lehner, Guy's and St Thomas's, London) is another dream on the way to coming true.

Vaccines, the classical tools of preventive medicine, naturally featured in the prospectuses of the 1970s, but people had not then thought of the use of vaccinia virus as a means of showing antigens chosen at will to the human immune system. Although there were several at last week's meeting sharing the caution expressed by Dr Geoffrey Schild (director of the British National Institute for Biological Standards and Control) about the potential of vaccinia virus and an all-purpose antigenic vehicle, the plain fact is that this material, the several versions of malaria vaccine now being prepared for trials and the vaccines being worked up as prophylactics of schistosomiasis (Dr André Capron, Pasteur, Lille) would not have been possible without genetic manipulation.

Inevitably, AIDS (Acquired Immune

Deficiency Syndrome) crops up in these connections. Who can but be moved to recognize that an infection whose viral character was first demonstrated merely three years ago now has an agent described almost as fully as more or less any other virus? Dr Luc Montagnier (Pasteur, Paris) gave such a telling account of this process of discovery last week, with due acknowledgement of the contributions of Gallo and his collaborators in the United States, that many in the audience must have come to share the view that it is a great misfortune that the French and US groups are now separated from each other by their patent suit.

Not surprisingly, AIDS brought into the open an issue lying beneath several of last week's talks. Montagnier had been talking about the rationale of a vaccine against AIDS, which is an awkward concept because it entails the stimulation of lymphocytes which, if already infected, will yield more virus. His chairman, Professor Avron Mitcheson, raised (by means of a reference to the case of yellow fever) the question whether physicians in earlier days might not have been impelled to see whether a vaccine would cure one or two of their patients otherwise destined for a terminal infection. Earlier Schild had remarked, in passing, that it would have been hard to win regulatory approval for the Sabin live-virus vaccine against poliomyelitis. How great an encumbrance is the regulatory apparatus?

To be fair, nobody seems concerned to sweep away the system that has grown up to regulate the introduction of new pharmaceuticals, for example; what people ask for there is greater efficiency and a plainer understanding on legal implications. (Does licensing imply immunity from some legal suits?) The regulation of new research is more disturbing. Dr French Anderson (National Institutes of Health, Bethesda), describing preparations being made for work with human beings, acknowledged that the present system is probably a necessary way of giving the general public reassurance — even though it will entail a delay of at least six months between application and consent. Anderson is almost certainly right that the public and governments would not be satisfied with less. But how quickly will the apparatus be dismantled when it has served its purpose, and when there is a real danger that regulation will become an impediment to therapy?

John Maddox

Taxonomy

A new class of echinoderms

from David Nichols

WATERLOGGED wood often sinks down to the deep sea-bed, to become an oasis of solidity in a vast expanse of sediment. Its crevices, filled with a soup of bacteria, become a haven for tiny organisms, including, it now emerges, a hitherto unknown echinoderm. So specialized are these 'sea-daisies' that a new class, the Concentricycloidea, has been erected to contain them. An article on page 862 of this issue describes the new genus, *Xyloplax*.

In August 1985, three echinoderm specialists, Alan Baker, Helen Clark and Frank Rowe, met at the National Museum of New Zealand principally to compare species of starfish common to waters off New Zealand and New South Wales. A worker at the museum, Bruce Marshall, was investigating gastropods from crevices in driftwood from a depth of 1,000 m and had put aside capsules with fragments or whole specimens of small caymanostelid starfishes for Rowe to examine during his visit. Sharing the capsules were nine specimens of a tiny animal, clearly an echinoderm, which Rowe and his colleagues could not place in any known group.

The disk-shaped body of the echinoderm is supported by a series of circumferential rings of plates (see Fig. 1) bearing a fringe of spines projecting like the petals of a daisy. The slightly domed dorsal integument contains scale-like plates that imbricate like roofing tiles, and each plate bears several stunted spines. Also visible on top are five large plates equally spaced around the periphery, representing the homologues of the plates at the armtips in starfishes, or those at the top of the radial columns (the so-called 'ambulacra') in sea-urchins.

Towards the circumference on the underside is a ring of hydraulic tube-feet projecting downwards. Each is apparently operated by a bag-like ampulla situated within the body, and all are connected externally by a water-vascular ring. Uniquely for echinoderms there is a second ring beside the first, and the two rings are connected by short inter-radial canals. In known echinoderms there is a functional need for confluence between the water-vascular system and the outside sea water, so that the hydrostatic pressure in the system equates with that of the outside medium. In *Xyloplax* too, this connection is provided at a hydropore on the dorsal

surface, connected to the inner water-vascular ring.

The animal has no gut, which takes to the ultimate the reduction in gut feeding of other echinoderms. Some sea-cucumbers, for instance, feed for only part of the year, generally when energy uptake is increased before reproduction. For the rest of the year, the gut remains empty and the organisms rely on the free radicals in sea water, absorbed mainly through a velvet-like 'pile' of microvilli over the external body surface.

The concentricycloids are reported to have a thin velum, which stretches drum-like across the ventral surface, and, further, the tube-feet themselves are thought to have food storage coelomocytes within their lumina. It is interesting to speculate that the suspension of bacteria which share the wood crevices with these animals may aid the nutritional process in a manner similar to that in deep-water pogonophores, which contain symbiotic bacteria in special tissues within their bodies. Perhaps in concentricycloids some bacteria are held beneath the sub-umbrella surface, within the palisade of spines, the radicals they release diffusing through the velum to the interior.

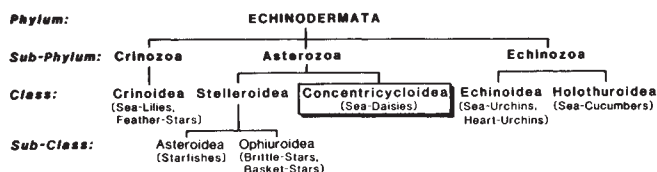


Fig. 2 Classification of echinoderms, showing possible relationships of the concentricycloids.

An exciting find is that the gonads of the animals contain embryos at various stages of development up to juveniles, which look like miniature adults. There are sound reasons for releasing young into the immediate vicinity of the adults when the 'parent' substrate is the only suitable habitat for a considerable distance. But timber, despite its slow decomposition in deep water, does not last for ever, and some mechanism is required for wider deployment of the offspring.

So long as the young concentricycloids are within the gonad, their peripheral spines are tucked under their bodies, but on release the spines open out, and are at this stage longer proportionally than the spines of the adult. This may be a flotation device to assist the juveniles to be carried above the sediment until they encounter another suitably pitted or creviced substrate.

As yet, it is possible to make only the

most tentative proposals about relationships of the new class. Suggestions by the authors that *Xyloplax* resembles the cyclocystoids (an extinct class of sub-circular echinoderms from the Middle Ordovician to Middle Devonian) may have been based on older reconstructions, now superseded by more detailed studies of almost all known cyclocystoid specimens (Smith, A.B. & Paul, C.R.C. *Phil. Trans. R. Soc. B* **296**, 577; 1982).

Cyclocystoids had a marginal ring of hefty plates with two plate-supported membranes stretched within. But the membranes were closely associated,

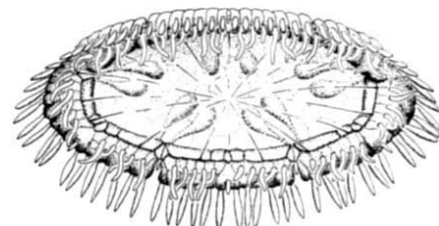


Fig. 1 Conjectural ventro-lateral view of *Xyloplax medusiformis*, a new concentricycloid from deep water off New Zealand.

dorsal to ventral, so that the organism would have resembled, as Smith and Paul put it, "a tambourine, not a drum", and the space between the membranes would have been very limited indeed, unlike the body-cavity in concentricycloids. Further, although earlier reconstructions (for example, Nichols, D. *Palaeontology* **15**, 519; 1972) suggested that a series of cupules in the marginal ring represent anchorages for a ring of tube-feet similar to the arrangement in *Xyloplax*, there are now grounds for rejecting this idea — first, the cupules have tubercles within them; and second, they do not connect up with the most likely seat of the water-vascular system, which provides fluid for tube-foot operation.

What, then, is the nearest known echinoderm relative of this new class? The authors suggest that it belongs to the Asterozoa, the sub-phylum that includes all starfishes and brittle-stars (see Fig. 2). This group already includes some pretty bizarre forms, such as the spherical starfish *Podosphaeraster* (Rowe, F.W.E., Nichols, D. & Jangoux, M. *Micronesica* **18**, 83; 1982; Rowe, F.W.E. *Bull. Mus. natn. Hist. nat., Paris* **7**, 309; 1985).

In the case of *Xyloplax*, a feasible derivation of the marginal ossicles is suggested by the authors, who see the ambulacral tube-feet, normally in a double column and radially arranged, as if having splayed out laterally and come to abut against the adjacent column in a circle. Clearly, a considered proposal about relationships awaits more rigorous study of this animal than has so far been possible. □

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Planetary magnetospheres

The double tilt of Uranus

from Fran Bagenal

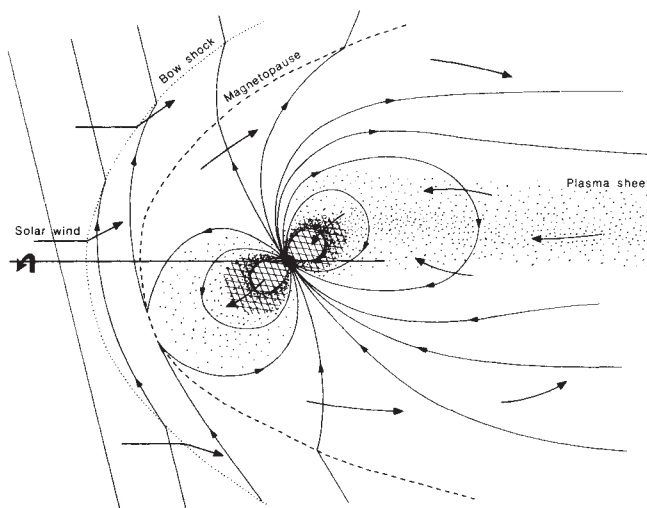
PERHAPS the biggest surprise of the Voyager 2 fly-by of Uranus was the discovery that the planet's magnetic field axis is tilted as much as 60° with respect to its rotation axis. Moreover, with Uranus tipped on its side, a highly tilted magnetic field produces a particularly unusual magnetosphere. The Voyager science teams have completed preliminary analyses of their data (published in this week's issue of *Science*) which were discussed at a recent meeting*.

The detection of strong ultraviolet emission from Uranus by the International Ultraviolet Explorer satellite in 1982 provoked speculation of a strong magnetic field, but it was not until Voyager 2 had reached a distance of 275 uranian radii ($1 R_U = 25,600$ km) from the planet (five days before its closest approach) that the detection of radio bursts established the existence of a uranian magnetic field. Approaching the planet near the sub-polar point the spacecraft first crossed the bow shock at $23.7 R_U$ and then entered the magnetosphere at $18.0 R_U$, where Voyager 2 discovered an unusually complex planetary magnetic field (N.F. Ness, Goddard Space Flight Center, GSFC). To first approximation the magnetic field can be described as a tilted 0.23 Gauss R_U^3 dipole. Higher order terms contribute as much as 70 per cent of the dipole terms and can be represented by displacing the dipole by $0.3 R_U$ from the centre of the planet away from the sunlit side. Consequently the surface field is much weaker on the sunlit side (<0.1 Gauss) than on the dark side (<1.1 Gauss). By combining magnetic field data with measurements of the periodic radio emission the rotation rate of the interior, dynamo region has been accurately determined at 17.24 ± 0.01 hours (see the paper by M.D. Desch, J.E.P. Connerney and M.L. Kaiser in next week's issue of *Nature*).

Once inside, Voyager 2 found the uranian magnetosphere to be relatively empty compared with Earth, Jupiter and Saturn. The total energy density of all trapped particle populations is much less than the magnetic field pressure ($\beta \ll 1$) and hence in the inner region ($\leq 12 R_U$) the magnetic field suffers very little distortion

from particle loading. Nevertheless for the few high-energy (few MeV) particles, fluxes were comparable to those in Saturn's radiation belts except absorption by the uranian satellites is much stronger (D. Chenette, Aerospace Corp.), which provoked the suggestion that particle bombardment of methane in the satellite's icy surfaces, producing tars, may be partly responsible for their dark appearance (L. Lanzerotti, Bell Laboratories).

The unusual depletion of energetic protons relative to the intense fluxes of electrons of similar energies may be due to preferential removal by charge-exchange reactions with the particularly extensive



Spots, hot plasma; hatching, cool plasma.

atomic hydrogen corona of Uranus (S. Krimigis, Johns Hopkins University). The density of low-energy thermal plasma was found to increase towards the planet, reaching a maximum value of $\sim 3 \text{ cm}^{-3}$ at closest approach (R.L. McNutt, MIT). The spatial distributions of the two plasma populations ($kT \sim \text{few keV}$ and $kT \sim 10 \text{ eV}$) provide clues to the nature of their sources and their subsequent transport: the cooler (10 eV) population was detected throughout the inner magnetosphere which suggests a source near Uranus (for example, its upper atmosphere or ionosphere), while a sharp inner boundary of the hotter population at $\sim 5 R_U$ indicates that hot material is brought in from distant sources (for example the solar wind or outer satellites) and prevented in some way from entering the inner region.

The Voyager 2 plasma measurements are consistent with the main plasma motion being that of co-rotation with the planet. In addition, the solar wind is

thought to drive a large-scale magnetospheric circulation, similar to that at Earth. Because plasmas are highly electrically conducting we can consider convective motions to consist of the movement of whole tubes of magnetic flux containing a parcel of plasma 'frozen' to the field. The solar wind carries flux tubes from the dayside magnetopause to the nightside. The flux tubes then follow a return flow through the centre of the magnetosphere towards the planet, heating any entrained plasma as the tubes contract. At Earth a point (the plasma-pause) is reached where atmospheric drag overpowers the magnetospheric forces and causes the plasma to rotate with the planet.

At Uranus the planet's rotation is orthogonal to the solar wind-driven flow (see figure) so that the two forces are orthogonal. Thus, if one were to watch a parcel of cool plasma it would appear to

move in a helical motion through the magnetosphere. Moreover, rotation of the whole magnetosphere guarantees that the planetary and solar wind magnetic fields are anti-parallel at the dayside magnetopause once per rotation. Hence periodic magnetic reconnection may allow the greater efficiency in removal of solar wind energy that is required to power magnetosphere in circulation plus auroral and electrogrow emissions (T. Hill and A.J. Dessler, Rice University).

Various features in the Voyager observations occurred at or were associated with magnetic field lines that cross the magnetic equator at a radial distance of $\sim 5 R_U$. This is also the orbital distance of the satellite Miranda, the small, icy body (~ 480 km diameter) whose bizarre surface features amazed planetary geologists. Could it be that this rather freakish object, like Jupiter's satellite Io, has a major impact on the magnetosphere? The Voyager science teams were quick to make such associations. Although the surface of Miranda shows a history of considerable geological activity there is no evidence of a means of producing material (such as Io's volcanic plumes) and at low temperatures it is unlikely that the satellite is electrically conducting.

Thus the truth may be that Miranda's role is no more exotic than that of other satellites, and that the moon is limited to sweeping up energetic particles. In particular, the $\sim 5 R_U$ boundary in the plasma data is more likely to be a convection boundary (J. Richardson, MIT). Inwardly increasing plasma densities and temperatures result in significant radial

* American Geophysical Union, Baltimore, Maryland, 19-22 May 1986.

pressure gradients. These essentially deflect the sunward flow and the flux tubes of hot plasma are swept around the inner region to the sunlit side. The plasma inside is then shielded from the electric fields associated with solar wind-driven circulation patterns. Alternatively one can think of the convection boundary being located where there is insufficient energy in the convective process to overcome the atmosphere/ionosphere drag (effectively electrical resistivity) at the ends of the flux tubes.

The solar wind deforms the outer magnetosphere ($>18 R_U$) 'downstream' of Uranus to produce a magnetotail (cylindrical radius $\sim 42 R_U$) extending behind the planet. The magnetotail is split into two lobes of oppositely directed field lines separated by a ($\sim 10 R_U$ thick) central plasma sheet which, as the rotation axis of Uranus is presently pointed essentially sunward, rotates with the planet every 17.24 hours.

Although not observed until the spacecraft was close to the planet, radio emission from Uranus is quite powerful (of the order of megawatts), between Earth and Saturn in emission strength and exhibits a complex spectral morphology with at least 5 different types of emission (M. Kaiser, GSFC). The peculiar geometry at Uranus may also explain why radio emission has not been detected from Earth since Larry Brown's observations in 1971. The most powerful radio bursts are thought to be beamed at low magnetic latitudes so that one would only detect uranian emission on Earth for a small fraction of the uranian 84-year orbital period. The next opportunity arises in 1994. At lower frequencies the plasma wave experiment detected local plasma waves (called Whistler-mode hiss and chorus), familiar from the magnetosphere of Earth, Jupiter and Saturn (F. Scarf, T.R.W. Inc.). These waves are thought to be in cyclotron resonance with energetic electrons which are then scattered by the waves so that they stream along the magnetic field lines into the planet's atmosphere, providing a source of energy for the nightside aurora.

The fundamental question of how the dynamo of Uranus can sustain such a rotational asymmetric magnetic field remains unanswered. Instead, a revised scaling law has been proposed (S. Curtis and N.F. Ness, GSFC) which predicts planetary magnetic fields based on the balance of coriolis and Lorenz forces in the core. A surface field of 0.5 Gauss is predicted for Neptune which would ensure a magnetic sphere large enough to encompass Triton. But after Uranus, serious predictions of the orientation of such a field are much harder to come by. □

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Oceanography

Predictability of El Niño

from S. G. H. Philander

THE unusually high sea-surface temperatures in the tropical Pacific Ocean during El Niño, and the associated major changes in the atmospheric circulation — which include devastating droughts in some regions and torrential floods in others — can cause economic and ecological disasters¹⁻⁵. Recent calculations indicate that some El Niño events could have been predicted several months in advance, either by statistical analysis of past data⁶, or by using a dynamical model to determine whether the inertia of the ocean at a given time predisposes it to certain developments⁷. On page 827 of this issue⁸, Cane, Zebiak and Dolan devise a model that extends predictability to approximately one year by taking interactions between the ocean and atmosphere into account. This relatively simple model appears remarkably successful, but its neglect of various physical processes could result in inaccurate predictions under certain circumstances. The predictions made during the first part of 1986 may turn out to be unreliable.

The changes in sea-surface temperatures and other oceanic conditions during El Niño are caused primarily by changes in the surface winds over the tropical Pacific⁹. A general circulation model of the ocean, forced with the winds that prevailed over the tropical Pacific during 1982 and 1983, realistically simulates all the major oceanic changes observed during that period¹⁰. The changes in the surface winds during El Niño are in turn part of the atmospheric response to the sea-surface temperature variations associated with the event. A general circulation model of the atmosphere realistically reproduces the interannual atmospheric variability (including the El Niño events) observed in the tropics between 1962 and 1976, provided observed sea-surface temperature variation in the tropical Pacific during that period are imposed as a lower boundary condition¹¹.

There have been similar simulations of the atmospheric changes during the El Niño of 1982–1983 (ref. 12). These circular oceanographic and meteorological results imply that interactions between the ocean and atmosphere are at the heart of El Niño phenomena. Such interactions have not yet been explored with the general circulation models I have just mentioned, but the use of much simpler models¹³⁻¹⁵ indicates that the interactions are unstable, as first suggested by Bjerknes¹⁶. Thus, a slight change in the atmosphere induces an oceanic response which in turn affects the atmosphere in such a manner as to reinforce the original

atmospheric change. Relaxation of the trade winds, for example, can cause warming of the central and eastern tropical Pacific which alters the atmospheric heating so as to cause a further weakening of the trades. This is how unstable air–sea interactions amplify a modest initial perturbation into an El Niño.

Once El Niño conditions prevail, another perturbation can initiate unstable interactions that reverse the warming trend, thus re-establishing low sea-surface temperatures in the eastern Pacific¹⁷. These unstable air–sea interactions depend on the manner in which sea-surface temperature variations affect the heating of the atmosphere. They are strongly modulated by the seasonal cycle because the seasonal movements of the atmospheric convergence zones determine when and where warm sea-surface temperature anomalies can cause a local heating of the atmosphere. It is for this reason that most El Niño events start as an amplification of the warm phase of the seasonal cycle in the eastern equatorial Pacific². The essence of El Niño is therefore the amplification of perturbations by unstable air–sea interactions which are modulated by the seasonal cycle. A simple stochastic model¹⁸ that incorporates these elements produces time-series in which El Niño events are as intermittent as in reality. (The dominant timescale is of the order of four years.) In this model the perturbations are assumed to be random and to represent atmospheric disturbances not attributable to interactions between the ocean and atmosphere. These include waves with a periodicity between 30 and 60 days observed in the tropics; disturbances that propagate into the tropics from higher latitudes; and variability induced

100 years ago

SMALL gelatinous masses that annually, about this time, cause the sea-water to become what fishermen call "foul", are now in great abundance on this coast. A pocket lens makes evident the presence of large specimens of the diatom *Eucampia britannica* that are seen as perfect spirals, some of which have four or five complete turns, and also some filamentous rods. Microscopic examination of the sediment deposited in the course of a few hours enables one to see *Rhizosolenia*, *Asterionella*, and several other diatoms whose names are not known to me. It will be interesting to know how far round our shores at the present time these organisms extend, and I hope, by thus again directing attention to them, that some one more competent than myself will be led to investigate their life history.

Sheerness

W.H. SHRUBSOLE

From *Nature* 34, 168; June 24, 1886.

by interannual changes in soil moisture and snow cover over continents.

A very different explanation for the irregular period between El Niño events invokes the long memory of the ocean as the principal source of perturbations¹⁹⁻²¹, assigning random atmospheric perturbations a minor role. In this model, the oceanic response to wind variations during El Niño does not end when the event ends but continues in the form of waves that slowly propagate westwards in off-equatorial latitudes. On reaching the western boundary of the ocean basin the waves give rise to renewed equatorial disturbances which, through unstable air-sea interactions, again amplify into a new El Niño. The interval between El Niño events is essentially the transit time of the waves, which in models of this type is of the order of three to four years. Some of the models^{19,20} are very periodic, but that of Cane and Zebiak²¹ is like an irregular nonlinear oscillator and produces time-series almost as aperiodic as those which are observed. But fluctuations with periods less than that of the seasonal cycle are far less energetic in the model than in reality because processes other than air-sea interactions that contribute to atmospheric variability are not considered.

The experimental predictions reported in this issue by Cane, Zebiak, and Dolan⁸ indicate that the atmospheric perturbations that are neglected in their model can cause predictions to diverge significantly after approximately one year. This is shorter than the timescale for the memory of the ocean (the waves) to initiate another event and suggests that the memory is not the dominant factor in determining the interval between El Niño events. The memory nonetheless is important in determining the predictability of the tropical oceans and atmospheres because it predisposes the ocean to behave in a certain manner. For a particular

model, the behaviour of the ocean at a certain time can be determined by keeping the winds constant from that time onwards: if the ocean is not in equilibrium with the winds then it has a memory of earlier wind variations and continues to adjust. Inoue and O'Brien⁷ find that if their model is forced with realistic winds up to April 1982 then El Niño starts to develop during the subsequent months even if the winds are held constant. In other words, the non-equilibrium state of the ocean in early 1982 predisposed it towards the development of El Niño.

The coupled air-sea model of Cane, Zebiak and Dolan⁸ simulates the air-sea interactions that amplify the oceanic tendency associated with its non-equilibrium state to provide a forecast of El Niño almost a year in advance. The predictability of the model is limited by its neglect of

atmospheric disturbances not attributable to air-sea interactions. These disturbances are particularly important when the ocean is in equilibrium with the prevailing winds and could mean that the prediction of an El Niño for 1986 is inaccurate. (Early in 1986, sea-surface temperatures in the eastern tropical Pacific Ocean were exceptionally high and El Niño appeared imminent. In mid-March the temperatures started to decrease and in May conditions looked essentially normal.) Reliable predictions require not only improved models of air-sea interactions but also a better understanding of and an ability to simulate the high-frequency atmospheric fluctuations. □

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Acid rain

Predictions and applications

from Nurtan A. Esmen

ACID rain, a controversial phenomenon which has attracted much recent attention, has nevertheless been ignored by many scientists who are interested in fundamental transport processes. Hence, experimental and theoretical studies of rain acidification have been more or less repetitious, albeit in different locations. Although some rigorous theoretical work has been done in the past decade, field verification of theoretical results has been at best incomplete. This scarcity results from the difficulties associated in measuring simultaneously the size and the acidity of raindrops using the conventional instruments of aerosol physics or meteorology. A new instrumental development reported by S.J. Adams *et al.* elsewhere in this issue (*Nature* **321**, 842; 1986), and the authors' preliminary measurements of raindrop acidity using the instrument, is good news for those interested in the problem of acid rain.

The new instrument, although ingenious, would be of limited interest if it were to apply only to one narrow area of research. Because the instrument, described in fuller detail elsewhere (Bradley, S.G. *J. Tech.* **2**, 190; 1985), can obtain good results under non-ideal conditions, it will be of interest in, for example, agricultural research on effective watering systems, chemical engineering and combustion equipment design.

The preliminary results of droplet size-dependent rain acidity reported by Adams *et al.* are intriguing. Besides the fact that they are the only such measurements that exist, and that they seem to verify qualitatively an existing theory of steady-state raindrop-gaseous pollutant interactions,

they also point to significant gaps in our understanding of both local and global influences on rainwater acidity. If the experimental results of Adams *et al.* are found in other sites, several hypotheses could be generated. For example, strong dependence of removal efficiency on raindrop size suggest that interactions between the mainly small drops of a drizzle and acid-producing gases such as sulphur dioxide would have significantly different consequences compared with the interaction between the same gas and the mainly large drops of a rain shower. Such an observation, if verified in different circumstances, opens up new areas of research into the effects of acid rain.

The results of Adams *et al.* also suggest that throughout a period of rainfall there is significant depletion of the local pollutant concentration. Thus, extended measurements using their new method may uncover a relationship between the variables of rain, in-cloud removal and below-cloud removal of contaminants. If such a relationship is found, past and new data can be corrected to factor-out local influences, allowing global transport of contaminants to be reanalysed on a uniform basis. Although we have a fairly good grasp of large-scale transport, important details are still missing and the predictive capacity of the available models is limited. The accident at the nuclear reactor at Chernobyl in the Soviet Union provides an example of the use to which such models, if accurate, could be put. □

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Archaean gold

Exploring for magmatic origins

from Neil Williams

ACCORDING to the 6 June issue of *Mining Journal*, gold mining is enjoying a period of accelerating growth, with the production of gold in the Western World reaching a 14-year high of 1,213 tonnes in 1985. This growth has triggered a high-technology gold rush and modern mineral explorers are retracing the steps of the old-time prospectors in the search for new El Dorados. Academics are also catching gold fever, but the findings of their research are often far from academic. For example, the report elsewhere in this issue by D.R. Burrows, P.C. Wood and E.T.C. Spooner (*Nature* **321**, 851; 1986) will leave many explorers wondering about the validity of some of the principles that are used in guiding their search for new gold deposits.

The article is controversial because it concludes that Archaean-age gold-quartz vein systems formed from magmatic fluids that originated from molten granite-like bodies deep beneath the surface of the Earth. This is contrary to the widely held view that the systems were deposited from metamorphic fluids, that is, from fluids that formed during the dehydration of wet sedimentary rocks as they were buried, heated and converted to metamorphic schists and gneisses. The controversy cannot be ignored because it surrounds the origin of a highly prized class of gold deposits that includes the main subject of the article, the Hollinger-McIntyre vein system in Ontario that produced nearly 1,000 tonnes of gold, as well as the fabulous Golden Mile system at Kalgoorlie, Western Australia, that has so far produced around 1,200 tonnes.

Why should someone seeking new Archaean gold-quartz vein deposits need to worry about the origin of mineralizing fluids that flowed through the Earth's crust more than two and a half billion years ago? To answer this question we must consider where new deposits are likely to occur, and how mineral explorers go about finding them. Most of the gold deposits discovered during the original gold rushes were exposed at the Earth's surface and were found because they had shed trails of alluvial gold that were easily traced by simple prospecting methods. Although these same methods still lead to an occasional discovery, most deposits not yet discovered have gone undetected because they are buried and have no surface expression.

The challenge in exploration is therefore to use methods that can unravel the subsurface geology of an area and accurately pin-point the position of buried

mineralization. Methods that are widely used today include satellite and airborne images that give a broad geological overview of the areas being explored; various airborne and ground-based geophysical methods that provide data on the magnetic, electrical and mineralogical properties of the rocks being investigated; and sensitive chemical tests that are able to detect the subtle chemical haloes that often envelop mineralization. However, none of these high-technology methods are of any value if the areas in which they are used were never mineralized, and to maximize the chances of discovery the explorer must therefore pay particular attention to selecting the most attractive ground possible. Many methods of ground selection may be used but nearly all rely to varying degrees on conceptual models.

These models attempt to describe, as accurately as possible, the sequence of events that leads to the formation of the type of mineral deposit being sought. The models bring together information from many different sources but are constructed primarily from empirical observations of known mineral deposits and theoretical and laboratory studies of the relevant ore-forming processes. The explorer uses the models to identify those geological features that are critical to the formation of the mineralization being modelled, and he or she tries to select areas for exploration that exhibit as many of the critical features as possible. If the model is accurate, it is clearly of great value in identifying the appropriate geological features, but if it is flawed and misidentifies a critical feature, subsequent exploration may be doomed to failure.

A typical model has three primary elements — a source for the mineralizing components, a site for the deposition of the ore minerals and a transport mechanism that transfers the mineralizing components from their source to the site of deposition. The metamorphic- versus magmatic-fluid debate being fuelled by Burrows *et al.* has an important implications for both the source and transport elements of exploration models for the Archaean quartz-gold vein deposits. If a metamorphic fluid is used it will lead explorers away from intensely metamorphosed terrains, where the fluids would have been generated, and into terrains of weaker metamorphism through which the fluids would have passed as they moved away from their source.

By contrast, if a magmatic-fluid model is used, it will lead explorers to seek out areas that are underlain by the appropriate granitic rocks. These may be the same terrains of weaker metamorphism that were highlighted by the metamorphic model, but they could equally well be the regions of intense metamorphism that would have been avoided using the metamorphic model.

Although we cannot be certain of the final outcome of the magmatic- versus metamorphic-fluid debate, we can be fairly certain that the article by Burrows *et al.* will not be the last word on the subject. As so often happens in stable isotope studies of mineral deposits, the carbon isotope data the authors use are likely to be reinterpreted by those favouring alternate views, and additional research will be needed to refine our understanding of Archaean quartz-gold vein mineralization and enhance our ability to explore efficiently for new examples of this important type of gold deposit. □

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Mathematics

Counting costs of calculation

from Ian Stewart

THE distinguished computer scientist Donald Knuth once remarked that a major difference between mathematics and computer science is that mathematicians do not, on the whole, worry about the cost of an algorithm — solution of a problem is paramount and the efficiency of the method is a secondary consideration. An entire new subject, complexity theory, has developed on the borders of mathematics and computer science to analyse just these questions. Until recently, the main concern of complexity theory has been discrete problems, although some

of the tools used have evolved from continuous mathematics. But now a parallel effort on the complexity of algorithms for continuous problems is appearing. A recent article by Steve Smale (*Bull. Am. Math. Soc.* **13**, 87; 1985) surveys this emerging area and goes so far as to suggest that a new concept of algorithm is required fully to understand such problems.

It has occasionally been suggested that the advent of computers will put mathematics out of business. Presumably the basis for this assertion is the idea that the computer can calculate anything that is

needed more quickly and more accurately than a human mathematician.

This is no doubt true but it has little bearing on the status of mathematics, because most interesting problems require a good deal more than brute calculation. On the contrary, the use of the computer raises new mathematical questions. The work already in existence exhibits a remarkable interaction between the theory of computation and both classical and modern mathematics. Computers enhance the capabilities of the mathematician but they are unlikely to render him or her obsolete.

Smale's survey begins by quoting a remark by Von Neumann made around 1950, to the effect that the then current theory of digital automata was "combinatorial rather than analytical" and calling for a "detailed, highly mathematical and more specifically analytical" theory.

Smale notes that although numerical analysis has made a strong contribution to such questions, it more often concerns itself with the asymptotic efficiency (for example, rate of convergence) of an algorithm than with its average efficiency or total cost. He says: "A study of the total cost for algorithms of numerical analysis yields side benefits. It forces one to consider global questions of speed of convergence, and in so doing one introduces topology and geometry in a natural way into that subject. I believe that this will have a tendency to systematize numerical analysis. This development could turn out to be comparable to the systematizing effect of dynamical systems on the subject of ordinary differential equations over the last twenty-five years."

Smale's previous work on topological dynamics has played a major part in the 'systematization' of differential equations that he refers to, and his remark is an understatement. The topological methods of dynamical systems theory have opened up a vast field of non-linear mathematics, currently a major growth area for research in several branches of science. A comparable development in numerical analysis would be of major importance.

The kinds of problem that Smale has in mind are drawn from numerical analysis, operations research and 'continuous' classical mathematics, for example, finding a zero of a polynomial of a system of non-linear equations. He cites one example which "a numerical analyst might give against the theoretization of his subject". The usual way to solve simultaneous non-linear equations is to pick an initial value at random (or by experience), use some iterative approximation method such as Newton's, and apply it for a while. If it doesn't work, pick a new initial guess and repeat, continuing until something does work. At first sight this does not look like a formal procedure amenable to rigorous analysis, but it is possible that a mixture

of probability theory and dynamics might be able to do this. Shub and Smale (*SIAM Journal of Computing*, in the press) show that for polynomials in one complex variable, this method requires on average only six random choices.

Another problem is linear programming — maximization of a given linear function of many variables subject to linear constraints. Consider a factory that makes different products subject to various costs trying to maximize profit by varying the quantities made. Until very recently the standard practical method for such a problem was an algorithm developed by George Dantzig, called the simplex method. In practice, it is observed that this algorithm 'usually' runs very efficiently, but that 'occasionally' it can take an enormous time to obtain an answer. Smale has made this observation precise, and proved it (*Math. Programming* 27, 241; 1983 and *Mathematical Programming, The State of the Art* (eds. Bachem *et al.* Springer, New York 1983). (A more recent algorithm, due to Kamarkar, holds the promise of running efficiently under all conditions.)

In elementary calculus, three standard methods of approximating integrals are often discussed: the Riemann sum; the trapezoidal method; and Simpson's rule. Smale's survey includes an analysis of these methods and their costs, confirming

the results of experience. On average, the trapezoidal method costs half as much as the Riemann approximation; Simpson's rule is much cheaper (in a sense that can be quantified precisely) than either.

A topic that is attractive both to the numerical analyst and to the topologist is Newton's method for finding a zero of a function $f(z)$. This takes a guess z and transforms it into $N_f(z) = z - f(z)/f'(z)$. If iteration of this procedure converges, the result is a good approximation to a zero of f . In fact, it does not always converge; this is related to the dynamics of the transformation N_f . A study of such phenomena, even when f is as simple a function as a polynomial, leads into deep and intricate 'pure' mathematics.

In closing, Smale singles out a key test problem for the field: what is the most efficient way to find zeros of a polynomial? He notes that merely to prove the existence of zeros of polynomials requires the development of complex numbers not only to find the zeros, but also to explain what manner of beast they are. A solution to the test problem similarly requires the development of a general concept of algorithm that, because of the way continuity is involved, may well go beyond the current combinatorial concept. □

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Geophysics

Looking for missing xenon

from Minoru Ozima

How can we determine the bulk chemical composition of the Earth? Analysing the right rocks tells us about the uppermost few hundred kilometres, and below this various indirect techniques can provide some constraints on the bulk physical properties, such as density. But for the past two decades, the composition of the Earth has been derived by analogy with that of meteorites. The method works well for most elements, with the notable exception of xenon. The usual solution to the problem of the missing Xe has been to propose some terrestrial sink such as shales¹ or ice². But successive laboratory work^{2,3} argues against this, and now Bernatowicz, Kennedy and Podosek hammer the point home in their report of measurements of Xe trapped in Antarctic ice⁴ — far too little exists to make up the missing xenon. Perhaps we should take another look at the meteorite analogy.

The method of meteorite analogy is based on the assumption that the Sun, planets, meteorite parent bodies and all solid materials in the Solar System condensed from the solar nebula, which was once heated to a vaporized state and was

therefore well homogenized. Hence, except for non-condensable volatile elements, the relative abundance of elements condensed on solid bodies is assumed to be quite similar to that in the original solar nebula. This assumption has been supported by observational data on the relative abundance of elements in the Sun's photosphere and by analytical data from meteorites. Once the meteorite analogy is accepted the bulk chemical composition of the Earth (at least of the condensable elements) can be equated to the abundance of elements observed in the solar photosphere and in meteorites (or, more precisely, in carbonaceous chondrites).

The noble gases, apart from Xe, are no exception. A remarkable resemblance between the abundance pattern of noble-gas elements in the atmospheres of terrestrial planets and in meteorites (see figure) is regarded as another successful example of the meteorite analogy. But the Xe discrepancy is usually attributed to some kind of sink, and has not been thought to violate the analogy. For example, Canals *et al.*¹ suggested shales as the sink: Xe is notoriously 'sticky' and can be easily ad-

sorbed in porous materials such as shale. But later experiments³, which examined both the trapped quantities of Xe in shales and the adsorptive capacities of shales for Xe, showed convincingly that this explanation does not account for the missing Xe.

Another suggestion was made by Wacker and Anders², who pointed out that because of its highly open structure and large abundance on the surface of the Earth, ice could form a Xe trap. But their own laboratory experiments² on adsorption of Xe by ice and the new measurements of Xe abundance in Antarctic glacial ice⁴ show that the concentrations of this element fall far short ($<10^{-3}$ in artificial ice and $\sim 10^{-4}$ in Arctic glacial ice) of the amount required for the meteorite analogy to hold. So where is the Xe?

One can still argue that much of the terrestrial Xe remains in the Earth's interior. But because the Xe content in volcanic rocks and in xenoliths is one to two orders of magnitude less than the atmospheric inventory (the total amount of Xe in the air divided by the mass of the Earth), the upper mantle is a very unlikely hiding place. This leaves the deeper mantle or core as the only possible sink.

Yet the whole problem stems from our original assumption of the analogy with meteorites. Perhaps we should therefore

question this assumption. It is known that atmospheric Xe isotope composition is distinct from that of the 'planetary' Xe observed in meteorites⁵, the latter being thought to represent primordial Xe in the Solar System. Such a large mass-dependent fractionation is very difficult to explain in terms of diffusion loss or gravitational escape. Even more difficult to explain is that although Xe shows such a large isotope fractionation (about 4 per cent per atomic mass unit), lighter noble gases show much smaller (argon) or even opposite (krypton) trends of fractionation. These observations indicate clearly that the origin of noble gases in the Earth is quite different from that in meteorites.

Doubts about the analogy are further augmented by space mission data (see figure) from the atmosphere of Mars: the abundancies of noble-gas elements are quite similar to those for the Earth's atmosphere, with a similar Xe deficiency. An interesting development in this story comes from the observation of Xe in a shergottite meteorite (EETA 79001)

which is probably from Mars; the Xe isotope composition is similar to the terrestrial content, but distinct from that of chondrites⁶. At present we do not know the extent of this disparity in the meteorites; but it seems clear that caution should be exercised in applying the meteorite analogy to the terrestrial planets. For example, we may need to question the longstanding 'meteorite game' in which certain types of meteorites are specified as the building blocks of the Earth, as no known meteorites have noble-gas element and isotope compositions that are reasonably related to those in the terrestrial planets. □

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GTP-binding proteins

One molecular machine can transduce diverse signals

from Henry R. Bourne

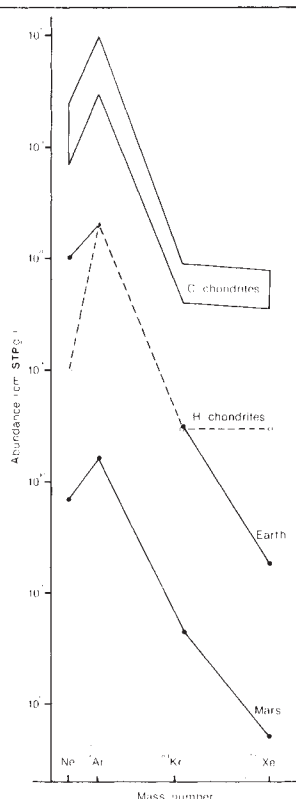
ARGUMENT by analogy, like gambling, was once practised behind closed doors. But the increasing recognition of duplicated and diverged genes has elevated it into respectability; it is still a gambler's strategy but, often enough, it works. A prime example of a rich payoff is the understanding of the single molecular machine that mediates vision, olfaction, control of cell proliferation, ribosomal protein synthesis and cellular regulation by a host of hormones and neurotransmitters. In each of its many forms, the machine is a guanine nucleotide-binding protein that mediates signal transduction through the following sequence of events. On stimulation by a receptor protein, it releases GDP and binds GTP. In its GTP-bound conformation, it regulates the function of an effector, usually an enzyme or ion channel. Hydrolysis of bound GTP to GDP terminates the regulatory effect of the G protein.

As was clear from a recent meeting* the power of molecular cloning and site-directed mutagenesis, combined with painstaking biochemistry and considerable luck, is revealing a rapidly expanding list of members of the three related families of GTP binding proteins: the G proteins; the *ras* proteins; and elongation factors (such as bacterial EF-Tu) and related

proteins involved in protein synthesis. Moreover, now that the innards of the GTP-binding machine are laid open to inspection, we can begin to understand its action (see bold text opposite).

The heterotrimeric G proteins contain three distinct chains — an α -chain that binds GTP and determines the specificity of the proteins for its detector and effector, and a β/γ complex that probably serves as an anchor to the cytoplasmic face of the plasma membrane. A recent frenzy of molecular cloning in many laboratories has identified complementary (c)DNAs encoding at least eight different α -chains, twice the number of biochemically purified G proteins. One of the latter, transducin, has been purified from outer segments of the photoreceptor rod cells in bovine retina. Oligonucleotides based on its amino-acid sequence led to isolation of not one, but two transducin α -chain (α_1) cDNAs. Antibodies directed against deduced α_1 peptide sequences reveal that one α_1 -chain is expressed exclusively in rods (responsible for night vision) and the other in cones, the colour detector cells (J. Hurley, University of Washington). In each case, activated transducin stimulates a cyclic GMP phosphodiesterase (PDE).

Molecular cloning now shows that the two different α -chains of G_{α_1} , a G protein that stimulates adenylyl cyclase in response to many hormones and neuro-



Noble gases in chondrites and planetary atmospheres. Planetary atmospheres closely match C-chondrites for Ne–Ar–Kr, but Xe is much depleted in Mars and Earth. C, carbonaceous; H, high-ion; solid circles, planets; open circles, chondrites. (He escapes from the planetary atmospheres and is not shown.) (Ref. 2.)

*G Proteins and Signal Transduction, 30 May – 4 April 1986, Cold Spring Harbor, New York.

Clues to the mechanism of action

The crystal structure of the guanine nucleotide binding domain of EF-Tu (F. Journak, University of California, Riverside; M. Kjeldgaard, Aarhus University) reveals a GDP molecule nestled in a partly open site that is bounded by a series of turns between β -strands and α -helices (see figure). Short stretches of amino-acid sequence in four of these regions, cross-hatched in the figure, exhibit close homology with sequences in p21 and the G protein α -chains; following Halliday's⁹ nomenclature, the regions are designated A, C, E and G. The bound GDP molecule appears to be fixed in position by the side chains of four key residues. Asp 80 (region C), which fixes the β phosphoryl via an interposed Mg^{2+} ion; Lys 24 (region A), also coordinated with the phosphoryls of GDP; and Asn 135 and Asp 138 (region G), which respectively bind the keto and amino substituents of the guanine ring. Replacement of GDP by GTP is thought to increase the distance between Asp 80 and residues that bind the guanine ring, enhancing the affinity of the protein for effector molecules.

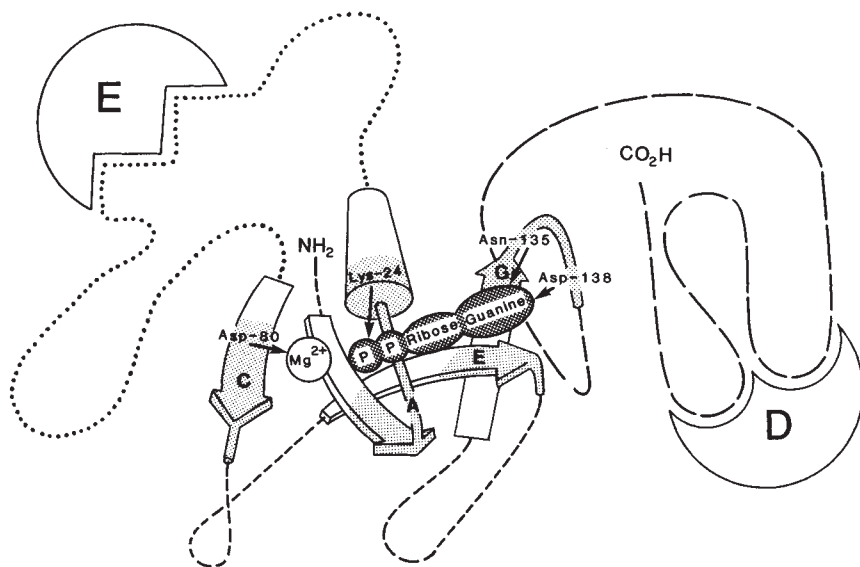
Extrapolated to p21, this picture rationalizes the effects of mutations that affect the handling of guanine nucleotides by the protein. Mutational replacements of Gly 12 in p21 (in the middle of the A region, near the phosphoryls of the bound nucleotide) were already known to stabilize the GTP-bound conformation of the protein to enhance its capacity for malignant transformation. Two additional mutations in p21 (I. Sigal, Merck Sharp & Dohme Research Laboratories) perturb binding of guanine nucleotides in different ways and also enhance its activity: replacement of the lysine corresponding to Lys 24 of EF-Tu by asparagine enhances the rate at which GDP is exchanged for GTP. Replacement by ala-

nine of the aspartate corresponding to Asp 138 of EF-Tu markedly reduces the affinity of p21 for both GDP and GTP, but increases its ability to stimulate yeast adenylyl cyclase, presumably by allowing the mutant protein to relax into an active conformation.

If binding of GTP induces an active conformation of the region that binds effector (E in the figure), where is the effector binding site located? Two clues suggest that it is situated somewhere between regions A and C (dotted line). First, the region between A and C in EF-Tu participates in the GTP-dependent binding of aminoacyl transfer RNA. Second, mutational replacement of residues in a short stretch (between positions 32 and 40 of p21) of the corresponding region of p21 reduces the biological effects of the protein without noticeably altering its known biochemical interactions with guanine nucleotides (Sigal). It is not yet known whether this region constitutes a

specific effector recognition site, and whether the much larger domain of the G protein α -chains (140 residues, compared with 40 or less in EF-Tu and p21) interacts with their respective effectors. Comparisons of the sequence of the putative effector region in p21 to deduced sequences in other *ras*-like molecules lead to the prediction (Sigal) that R-*ras* and the p21 products of the three known *ras* oncogenes will be found to interact with a common set of effector molecules, but that *ral* and *rho* will not.

It is attractive to postulate that the detector (D) binding regions of p21 and the G protein α -chains are distal to the G turn in the guanine nucleotide binding site (long dashes). The carboxyterminal 25 residues of the α -chains are a strong candidate for a detector binding region. This region, which I reported to have the predicted secondary structure of an amphipathic α -helix, contains the cysteine whose modification by pertussis toxin prevents coupling of G_i and transducin to their respective receptors.



transmitters such as β -adrenergic amines, result from alternative splicing of the transcript of a single gene (A. Gilman, University of Texas). G_i probably mediates olfactory transduction as well, as odorant stimuli stimulate GTP-dependent cyclic AMP synthesis in olfactory epithelia of the rat (R. Reed, Johns Hopkins School of Medicine) and the frog (D. Lancet, Weizmann Institute). Further evidence for the role of G_i in olfaction comes from a study¹ of patients with pseudo-hypoparathyroidism (PHP), an inherited disease of hormone resistance caused by a generalized partial deficiency of G_i activity^{2,3}. Compared with normal subjects, each of five G_i -deficient PHP patients are unable to identify common odorants¹.

Complementary DNAs encoding α -chains of two other purified G proteins, G_o

and G_{12} , have been cloned. G_i mediates inhibition of adenylyl cyclase by receptors for several hormones and neurotransmitters (for example, muscarinic agonists and somatostatin) and is a substrate for pertussis toxin-catalysed ADP-ribosylation, a covalent modification that uncouples G_i from its receptors. Another substrate is G_o , the predominant G protein of mammalian brain. The function of G_o is unknown, except that it can interact with muscarinic receptors *in vitro* (P. Sternweis, University of Texas). Now, comparisons of cDNAs from several laboratories indicate that bovine brain (T. Nukada, Kyoto University; E. Neer, Harvard University) and C6 rat glioma cells (Y. Kaziro, University of Tokyo) express, in addition to α_i , at least two distinct α_o -chains. Furthermore, a cDNA library derived

from rat olfactory epithelium contains two cDNAs encoding α -chains not yet identified with any known G protein (Reed).

The unexpected cornucopia of α -chains challenges investigators to determine whether they transduce signals to different effectors. Two prime candidates are ion channels, such as the cardiac K^+ channel that is regulated by ligands via a pertussis toxin-sensitive G protein (G. Szabo, University of Texas) and phospholipase C (PLC), the enzyme that triggers the phosphoinositide cascade which results in increased intracellular Ca^{2+} and activation of protein kinase C (ref.4). G proteins that regulate PLC are sensitive to pertussis toxin in some tissues. Recombinant-DNA produced α -chain polypeptides and chain-specific antisera will help to assign G proteins to specific functions.

Evolution has also produced variants of the other two chains of the G protein heterotrimer. Complementary DNAs from bovine retina (M. Simon, Caltech) and brain (Nukada) predict the primary structure of a β -chain, but fail to account for an immunologically distinct β -polypeptide found in most tissues (but not in rod outer segments). A cDNA encoding the γ -chain of transducin hybridizes to a messenger RNA found only in the retina (Simon) but in other tissues there are at least two more γ -chains, distinguishable immunologically and in apparent size (T. Evans, University of Calgary). Molecular cloning will soon establish whether distinct genes encode the multiple β - or γ -chains. If the sole function of β/γ is to anchor the G protein to the membrane, it is puzzling that there should be two or three versions of each polypeptide.

The *ras* protein family includes two *RAS* proteins that control adenylyl cyclase in yeast and the three p21 proteins that are the products of the mammalian *ras* proto-oncogenes *Ha-*, *Ki-* and *N-ras*. Nucleic acid hybridization techniques have recently discovered at least three kinds of *ras*-related proteins, whose functions, like those of several G protein α -chains, are unknown. They include, in descending order of similarity to the known *ras* proteins: human *R-ras*, which has a 26-residue amino-terminal extension not seen in p21 (D. Lowe, Genentech); *ral*, identified in a simian lymphocyte cDNA library (P. Chardin, INSERM); and a subfamily (three genes in man, two in yeast) of *rho* proteins (P. Madaule, Columbia).

Experiments reported by A. Hall (Institute of Cancer Research, London) provide a strong hint that the *N-ras* protein couples receptors for polypeptide agonists to activation of PLC. The date will be published in, and separately commented on, in a subsequent issue of *Nature*.

Of the G protein effector enzymes, PLC is turning out to be harder to handle biochemically than adenylyl cyclase and the PDE of retinal rods, both of which have been purified and functionally reconstituted with the appropriate G proteins and detector components. Fortunately, *Saccharomyces cerevisiae* and the power of yeast genetics may come to the rescue. Feeding glucose to glucose-starved yeast cells causes rapid activation of PLC, production of inositol trisphosphate, and mobilization of intracellular calcium (K. Kaibuchi, DNAX Research Institute). Glucose metabolites, rather than glucose itself, may be the relevant agonists, because non-metabolizable glucose analogues produce no effect. Although preliminary experiments suggest that activation of yeast PLC is GTP dependent, the PLC response is augmented rather than abolished in cells lacking the *RAS* proteins.

Genetic analysis has already elucidated

many key features of the yeast adenylyl cyclase signalling system, establishing the G_i-like role of the *RAS* proteins and identifying a potential detector protein, the *CDC25* gene product, which supports Mg²⁺- and guanine nucleotide-dependent regulation of cyclic AMP synthesis (D. Broek, Cold Spring Harbor Laboratory). Elegant manipulation of the yeast adenylyl cyclase gene (T. Kataoka, Harvard University) has also identified separate catalytic and *RAS*-regulated domains of the catalyst.

Finally, molecular cloning and biochemistry reveal some surprising common structures and turn-off mechanisms among detector components that control mammalian G proteins. The cloning of cDNAs that encode β -adrenoceptors (β -AR), from the hamster (R. Lefkowitz, Duke University)⁶ and the turkey (E. Ross, University of Texas; Y. Yarden, Genentech) opens avenues to understanding detector structure and mechanism. Hydrophobicity plots of the deduced primary structures of the two β -ARs suggest a common overall structure, shared with retinal rhodopsin⁷, in which each protein chain weaves through the plasma membrane seven times, with its carboxy terminus in the cytoplasm and its amino terminus outside.

One would have expected strong conservation of the amino-acid sequence in the putative extracellular and cytoplasmic regions of the two β -ARs, since both mediate interactions with G_i and catecholamines; instead, the transmembrane regions are much more highly conserved than the hydrophilic tails and loops of the two receptors. Because proteolytic studies of rhodopsin show that its third cytoplasmic loop is essential for coupling to transducin (H. Kuhn, Institute for Neurobiology, Jülich), by analogy it is unlikely that the hydrophilic regions of β -AR are irrelevant for signalling between extracellular agonist and intracellular G_i. But perhaps some portions of the agonist or G_i molecules interact directly with hydrophobic intramembrane regions of β -AR, and perhaps intimate contacts in the conserved transmembrane domains are crucial in signalling.

The β -AR also shares with rhodopsin a common biochemical turn-off mechanism, as reported by Benovic *et al.* on page 869 of this issue⁸. Photoexcited rhodopsin (but not dark rhodopsin) was known to be a substrate for phosphorylation by rhodopsin kinase. Now Benovic *et al.* show that an analogous β -AR kinase also phosphorylates photoexcited rhodopsin, probably on the multiple serine and threonine residues that are phosphorylated by rhodopsin kinase in the carboxy-terminal tail of the photon detector protein. To emphasize the homology further, rhodopsin kinase can also phosphorylate β -AR. Each kinase shows some preference for its

own signal detector as substrate but both vastly prefer activated detectors (photo-rhodopsin or agonist-bound β -AR), even in the heterologous reactions.

In retinal rods, phosphorylation of rhodopsin by its kinase promotes its binding to a protein (arrestin or S-antigen) which turns off the photon signal by preventing the detector protein from interacting with transducin. The β -AR kinase probably triggers a similar turn-off of the catecholamine signal, as suggested by a temporal correlation in intact cells between β -agonist-induced phosphorylation of the β -AR and agonist-induced desensitization of the β -adrenergic response. To push the analogy further, is an arrestin-like protein required for turning off phosphorylated, agonist-bound β -AR? Preliminary evidence suggests that the answer is yes: in reconstitution experiments, β -AR kinase-catalysed phosphorylation attenuates coupling of β -AR to G_i; the effect decreases with increasing purity of kinase, however, suggesting that the purification procedure is separating the kinase from an analogue of arrestin (Lefkowitz).

Throughout the meeting, argument by analogy proved a dominant theme. An ideal test for the power of such an approach was provided by the report of a new human oncogene, *mas*. The *mas* open reading frame encodes a protein with a hydrophobicity plot that closely resembles that of rhodopsin and β -AR, suggesting that the protein has seven transmembrane domains (D. Young, Cold Spring Harbor Laboratory). Analogy spins from these observations a gossamer web of hypotheses: first, the *mas* protein is a cell surface receptor, like β -AR; second, because its expression produces changes associated with malignant transformation, *mas* detects an extracellular ligand that promotes cell proliferation; third, because its structure resembles those of rhodopsin and β -AR (seven is a powerful cabalistic number), *mas* works by triggering a GTP-binding protein, probably a G, but conceivably a *ras*, protein; and, finally, the GTP-binding protein of *mas* stimulates an effector protein, possibly PLC, that generates second messenger molecules within the cell. Who will bet that *mas* will not fulfill many of these predictions? □

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Agricultural impact of Chernobyl: a warning

SIR—Since the evening of 30 April 1986, when a series of thunderstorms brought down heavy fallout from Chernobyl along the northern flank of the Alps, we have conducted high-resolution γ spectroscopy measurements on rain water, on a high-efficiency particulate air (HEPA) filter and on various agricultural products from the region around Konstanz (West Germany). Our results on the purely physical aspects of the fallout are comparable to those already published^{1,2}. Specifically, from the activity ratio $^{103}\text{Ru}/^{106}\text{Ru} = 4.5(5)$ and the published values of fission yields, we estimate the age of the Chernobyl core to be 2.0(5) yr. In addition, from a detailed analysis of activities, we infer relative release fractions in six of seven volatility classes and conclude that Chernobyl released core activity in the same proportions as the extensively documented 'reference accident' (PWR2)^{3,4}. This putative accident involved core melting and complete loss of containment of a 1,100-MW (electric) light-water reactor. Our observations are therefore consistent with a core melt at Chernobyl.

In the course of monitoring agricultural products and ground activity around Konstanz, we have noted three aspects of Chernobyl fallout that may have considerable practical importance for agricultural management, both in strongly contaminated regions close to Chernobyl and in many parts of Western Europe.

(1) Fallout from Chernobyl shows extreme geographical variability over short distances, depending on rainfall patterns prevailing when the cloud passed. For example, within the West German state of Baden-Württemberg, a region of $\sim 175 \times$

225 km, gross β activity at ground level, determined uniformly, varied by up to 30-fold (data from the Landesregierung Baden-Württemberg).

(2) Fallout aerosols brought down by rainfall show a high resistance to weathering and drying. In experiments we found that grass dried at 130°C for 24 h, followed by vigorous shaking, lost less than 10 per cent of the adhering ^{131}I , ^{132}I / ^{132}Te , ^{134}Cs , ^{136}Cs , ^{137}Cs , $^{140}\text{Ba}/^{140}\text{La}$, ^{99}Mo , and ^{103}Ru (Fig. 1).

(3) Cutting, drying and storing grass for winter cattle feed will therefore lead to radioactive accumulations in silos and barns. Near Konstanz, where ground activity includes $\sim 10^4\text{ Bq m}^{-2}$ of ^{137}Cs (half-life ≈ 30 yr), direct measurement and calculations lead us to expect $\sim 0.2\text{--}5\text{ mCi}$ of ^{137}Cs in typical small-farm storage facilities. Further east much larger ground activity has been reported and correspondingly larger accumulations are likely.

According to radiation protection laws in most countries, transport and storage of such quantities requires user licences that are normally reserved for a few well-equipped scientific and technological institutions. From a health perspective, the expected accumulations will contaminate barns, lead to significant exposure of farm workers and may pose special threats to children.

The problem of hay accumulation and storage may be all the more acute in view of the recent discovery¹ that fallout aerosols contain $1\text{--}2\text{-}\mu\text{m}$ 'hot' β -emitting particles with single-isotope source strengths of $1,000\text{--}10,000\text{ Bq}$. If these are widespread constituents of Chernobyl fallout, they can be expected to be trapped 'permanently' in the lungs of individuals exposed to contaminated hay.

To our knowledge the biological effects of such hot β -emitting particles are not

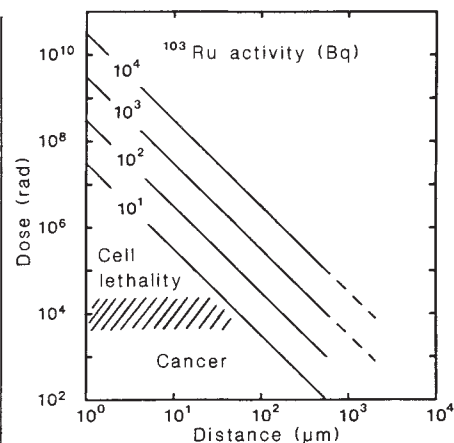


Fig. 2. Local radiation dose as a function of distance from a $1\text{-}\mu\text{m}$ -diameter hot β -emitting particle of ^{103}Ru (half-life ≈ 40 days). The dose assumes integration of the activity over many half-lives, as is appropriate for permanent lung deposition of the particle.

well described. Average dose calculations may not be valid for predicting consequences. Figure 2 shows the results of a sample dose calculation for ^{103}Ru particles of various strengths, and implies a substantial zone of cell lethality at short distances followed by an annulus of very high but non-lethal dose at larger distances. Compared with α -emitting hot particles, the lethal and non-lethal volumes are orders of magnitude larger.

We thank H. Haas of the Hahn Meitner Institute (Berlin) for discussions and the many colleagues and students who participated in recent measurements.

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First assessment of Chernobyl radioactive plume over Paris

SIR—Measurements of the gamma-radioactivity of the surface air in Paris after the Chernobyl accident have shown that the maximum concentrations of ^{137}Cs over a period of three days were 400 times greater than during the worldwide fallout of 1963. Preliminary estimates of ground deposition of ^{137}Cs , amounting to between 2 and 41 mCi km^{-2} (or between 74 and 1,500 MBq km^{-2}), suggest that the recent

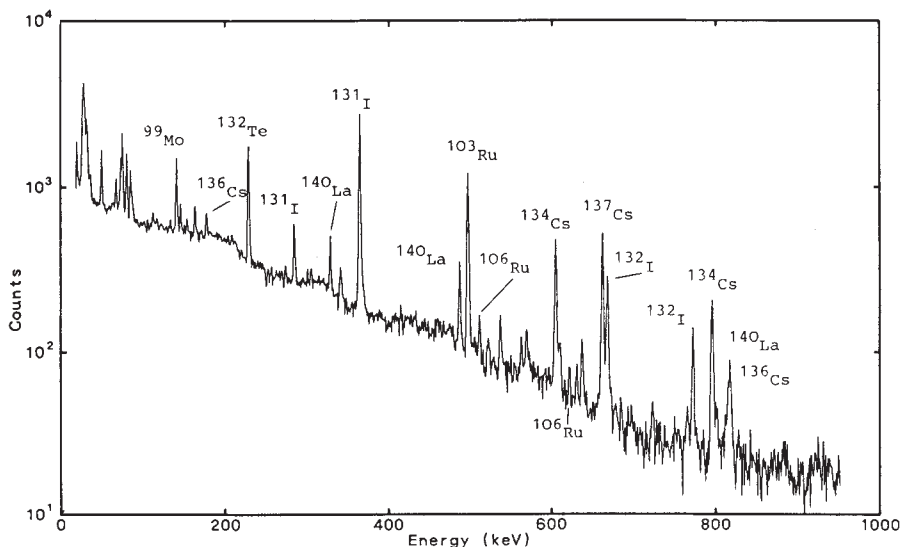


Fig. 1. Gamma-ray spectrum of grass recorded on 10 May 1986 showing dominant isotopes present in adhering particulates.

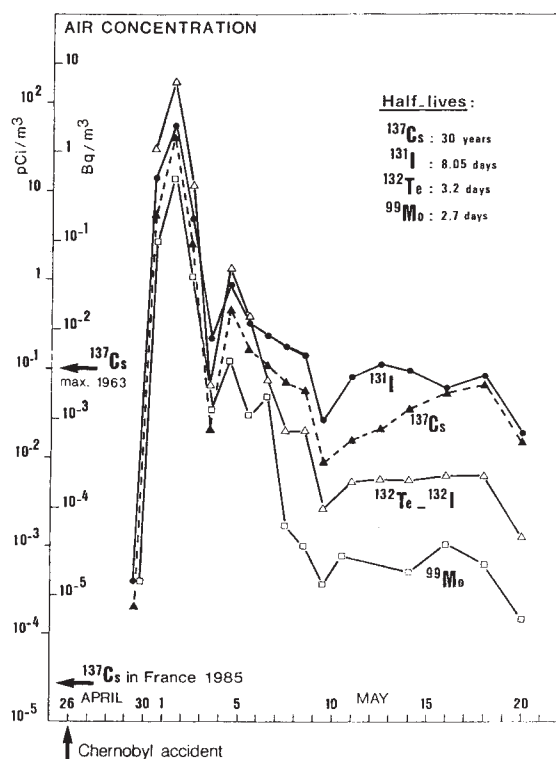


Fig. 1 Concentrations of selected artificial γ -emitters in air filters at Paris.

event is probably comparable in its effects with the global fallout of 1963.

The radioactive plume was first detected over Paris on 29 April (Fig. 1); twenty short- and long-lived nuclides have been detected, and the peak activity (for ^{132}Te) was measured at 170 pCi m^{-3} (6.3 Bq m^{-3}). After three days, the influx of marine air followed by atmospheric washout led to a reduction of the atmospheric activity by three orders of magnitude in the following week. Although some official data were released in France on 8 May, no detailed account of the radionuclide composition of the fallout has previously been published.

In the days after the Chernobyl accident, this institute intensified its routine programme for monitoring air and rain composition. Ground-level air was filtered daily (at $110 \text{ m}^3 \text{ s}^{-1}$) through Whatman EPM-1000 glass-fibre filters. A rain sample was also collected in a pre-acidified plastic bottle coupled to a 1-m^2 collector and was reduced for gamma spectroscopy to a volume of 30 cm^3 . (The slow evaporation used in this treatment probably causes some loss of iodine, but experience has shown that the analysis for other materials is reliable. Similarly, the high percentage of gaseous or desorbable iodine¹ means that the measurements of the activity collected on particle filters probably underestimate the atmospheric content of radioiodine by a factor of between 2 and 5.) Gamma-spectroscopy was carried out with a low-level, high-purity Ge detector (with 32% efficiency and sensitivity of 1.8 keV at 1.332 keV).

Unusual radionuclides (^{99}Mo , ^{131}I) were first detected in the air filters on 29 April.

Maximum concentrations were reached between 1 and 2 May, coinciding with the maximum of atmospheric dust (0.11 mg m^{-3}). Between 2 and 4 May, the concentrations of all the measured radionuclides decreased in parallel, at a rate independent of their half-lives, with the arrival of an Atlantic air mass and the rainfall (0.1 mm) associated with a small storm on 3 May, when the atmospheric dust content fell to 0.02 mg m^{-3} . The general decline of activity continued thereafter until 9 May.

The increase in the activity of the long-lived nuclides (^{137}Cs , ^{103}Ru , ^{125}Sb) that we observed between 10 and 19 May probably represents a second passage over Paris of the radioactive plume, with its radioactive content reduced by three orders of magnitude, after a circumnavigation of the Earth. We note, however, that this secondary maximum was not pronounced in respect of ^{99}Mo , ^{132}Te and ^{131}I . Thereafter, atmospheric activity decreased steadily until 21 May, when we ceased our daily monitoring. Throughout our observations, the concentration of cosmogenic ^7Be remained constant at $82 \pm 22 \text{ fCi m}^{-3}$ ($3.0 \pm 0.8 \text{ mBq m}^{-3}$).

While the largest activities were those corresponding to volatile elements such as iodine, tellurium and caesium, the high concentration of non-volatile elements confirms previous indications¹ that the temperature reached in the reactor must have been very high. Apart from the radioactive elements ^{110}Ag , ^{140}Ba , ^7Be , ^{141}Ce , ^{144}Ce , ^{134}Cs , ^{136}Cs , ^{137}Cs , ^{131}I , ^{99}Mo , ^{103}Ru , ^{106}Ru , ^{125}Sb , $^{129\text{m}}\text{Te}$, ^{132}Te and ^{132}I , we also detected short-lived isotopes such as ^{137}U and $^{131\text{m}}\text{Te}$, with activities of 6.3 and 3.1 Bq m^{-3} respectively, and long-lived

isotopes such as ^{95}Zr , ^{95}Nb and (probably) ^{51}Cr and ^{124}Sb appeared in the gamma spectra after the decay of the most active short-lived nuclides. Further counting will allow us to make a more accurate determination of the nuclides involved. Although we were not able to arrive at an accurate estimate of the $^{140}\text{Ba}/^{140}\text{La}$ activity ratio before equilibrium had been reached, data from early filters confirm the calculation of ref. 1 that the reactor was shut down on 26 April.

During our observations, the radioactive nuclides detected increased and then declined in line with each other. All activities reached a maximum during the period of 23.48 hours spanning 1 and 2 May, but the levels of all the radionuclides increased significantly between the samples collected on 4 and 5 May, usually by a factor of 4 or greater (Fig. 1). This accords with the belief that the original radioactive plume, diluted with the passage of time, was carried over Paris on that second occasion.

Our evidence in support of the suggestion in ref. 1 of the presence of ^{239}Np among the nuclides is more equivocal. Gamma peaks were detected in the 99–106 keV range, but there are many possible sources of interference. Accordingly, we have sought evidence for the presence of the daughter product, ^{239}Pu , by alpha spectroscopy. Our preliminary results suggest that the total concentrations of ^{239}Pu and ^{240}Pu are less than 0.004 mBq m^{-3} . It has separately been reported² that during 1984, total $^{239}+^{240}$ activity was much smaller ($10\text{--}40 \text{ nBq m}^{-3}$), so that our preliminary data do not exclude the possibility of a significant enrichment of ^{239}Pu in the air at Paris.

During the sampling period, the activity ratios of pairs of isotopes of the same element showed only small fluctuations about their decay slopes, which has allowed us to calculate the initial ratios $^{132}\text{Te}/^{129\text{m}}\text{Te}=11$; $^{135}\text{Cs}/^{137}\text{Cs}=0.21$; $^{141}\text{Ce}/^{144}\text{Ce} \approx 1.4$; $^{103}\text{Ru}/^{106}\text{Ru}=5.4$; and $^{134}\text{Cs}/^{137}\text{Cs}=0.506$. These ratios should provide useful tracers of Chernobyl fallout in future environmental studies.

Deposition during the period of maximum air concentration was estimated from the sample of rain water collected over a period of 86 hours between 29 April and 3 May. Activities in the sampled liquid were particularly high, amounting to $7,400 \text{ Bq per litre}$ for ^{132}Te and 700 Bq per litre for ^{137}Cs , for example. We did not attempt to estimate the contribution of dry deposition to these data.

The significance of our measured values of activity of long-lived nuclides such as ^{137}Cs can be estimated by comparison with the maximum atmospheric concentration reached in 1963, after the cessation of major nuclear bomb tests in the atmosphere. Then, ^{137}Cs concentrations at $46^\circ 07' \text{ N}$ reached 3.7 mBq m^{-3} (see ref. 3), less by two orders of magnitude than the

maximum due to the Chernobyl accident at Paris of $1,480 \text{ mBq m}^{-3}$.

Deposition at ground level at Paris during the Chernobyl peak (between 29 April and 3 May) was measured as 74 mBq km^{-2} . Between 29 April and 19 May, a total of $51,786 \text{ m}^3$ of air passed through the filters, giving an average concentration of 90 mBq m^{-3} . During this period, average rainfall was low (18.1 mm). If we assume that the usual deposition velocity of $\sim 1 \text{ cm s}^{-1}$ applies, ground deposition at Paris would amount to $1,500 \text{ MBq km}^{-2}$, although it is clear that this result must depend on local weather conditions. In 1963, the average deposition² of ^{137}Cs throughout the Northern Hemisphere was $\sim 590 \text{ MBq km}^{-2}$. Although this estimate needs to be confirmed by continuing measurements of ground deposition, it seems likely that ^{137}Cs deposition at Paris after the Chernobyl accident will be comparable to that during 1963.

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High level radioactive nuclides in Japan in May

SIR—The accidental release of radioactive materials from the Chernobyl nuclear power plant on 26 April 1986 is known to have caused significant pollution in Western Europe at distances as great as 2,000 km. We have been measuring natural and artificial radionuclides in the environment of Japan for the past three decades, chiefly with a network of eleven monitoring stations, from Wakkanai (45.4°N) to Ishigaki (24.3°N), established for the assessment of the effects of atmospheric nuclear bomb tests.

After the detection of radioactivity in Sweden on 28 April, we began to monitor radioactivity in air and water samples. On 3 May, a week after the accidental release, the radioactivity of the surface air and water abruptly increased in the middle of Honshu Island.

Precipitation, airborne particles and gases were collected at the observing station of the Meteorological Research Institute, Tsukuba. Precipitation was collected by a 1-m^2 collector at every precipitation event. Initially, 50-cm^3 portions of the rain were sufficient for the measurement of radioactivity; radioiodine was

Table 1 Activity concentrations in air near ground level at Tsukuba (pCi m^{-3})

Sampling date	Volume (m^3)	^{137}Cs	Radioactive nuclides			
			^{134}Cs	^{131}I	^{132}I (^{132}Te)	^{103}Ru
26 Apr. – 30 Apr.	5,600	0.00037				
30 Apr. – 3 May	4,600	ND				
3 May – 5 May	2,880	0.64	0.33	4.70	1.65	1.28
5 May – 6 May	1,300	1.53	0.86	6.27	2.60	2.42
6 May – 7 May	1,300	0.50	0.26	2.41	0.76	1.05
7 May – 8 May	1,400	0.87	0.43	3.02	1.01	1.69
8 May – 9 May	1,400	1.46	0.74	4.08	1.36	2.77
9 May – 10 May	1,400	1.64	0.83	3.89	1.47	3.44
10 May – 11 May	1,380	0.09	0.04	0.40	0.04	0.09
11 May – 12 May	1,420	0.25	0.11	0.94	0.10	0.36
12 May – 13 May	1,320	0.16	0.08	0.88	0.06	0.31
13 May – 14 May	1,370	0.28	0.13	1.15	0.10	0.46
14 May – 15 May	1,360	0.27	0.14	0.80	0.07	0.39
15 May – 16 May	1,290	0.00	0.01	0.11	0.00	0.01
16 May – 17 May	1,330	0.07	0.02	0.31	0.00	0.11

Sample was changed at 9:00 a.m., and radioactive decay was corrected to the middle of the sampling period. ND, not determined.

converted to silver iodide by wet-chemical methods.

Airborne particles were collected on glass-fibre filters (Toyo GB100R, nominal efficiency 99.9% at $0.3 \mu\text{m}$, flow rate 1,000 litres per minute). Filters were changed every morning. Airborne radioactivity, gaseous as well as particulate, was also collected by means of an active charcoal trap (30–60 mesh, 24 mm diameter, 85 mm long, flow rate 8 litres per minute). ^{85}Kr was estimated by cryogenic separation from air samples collected at intervals in an iron cylinder (14 litres, 120 atmospheres) followed by gas-chromatographic purification¹.

Before the abrupt change of atmospheric radioactivity, the concentration of ^{137}Cs ($T_{1/2} \approx 30 \text{ yr}$) in the air derived from continuing stratospheric fallout was 0.4 fCi m^{-3} or $14.8 \mu\text{Bq m}^{-3}$. (In what follows, units of activity have been converted from Ci to Bq by the factor $1 \text{ Ci} = 3.70 \times 10^{10} \text{ Bq}$.) In the filter sample of 3–5 May, the concentration had increased abruptly to 0.64 pCi m^{-3} , an increase of about a thousandfold, while significant amounts of other radionuclides were detected in the sample. (These were ^{131}I , ^{132}Te , ^{132}I , ^{103}Ru , ^{106}Ru , ^{106}Rh , ^{134}Cs , ^{144}Ce , ^{140}Ba , ^{140}La , ^{99}Mo and $^{99\text{m}}\text{Tc}$.)

The highest concentrations of radioactivity (Table 1), to which ^{131}I was the chief contributor, were found in the sample collected between 5 and 10 May, and persisted for several days. The ^{131}I concentration was about a fiftieth of that observed at Studsvik, Sweden². In the period 3–15 May, the activity ratio $^{137}\text{Cs}/^{134}\text{Cs}$ was 2.0 ± 0.3 , in good agreement with that observed at Studsvik. Airborne radioactivity decreased with an apparent half-life time of three days, until it decreased abruptly after a precipitation event.

The size distribution of particles carrying ^{131}I activity showed a peak at $0.43 \mu\text{m}$ aerodynamic diameter, and more than half of the total was present in particles smaller than this. Iodine is injected into

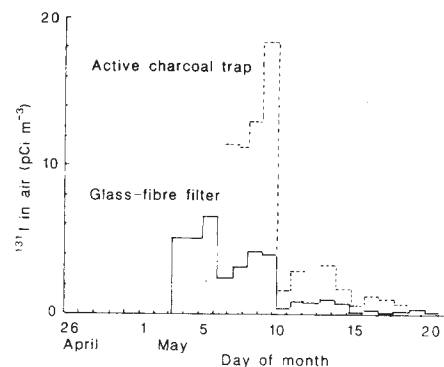


Fig. 1 The ^{131}I concentrations captured by the active charcoal and in the filters.

the atmosphere in gaseous form, and it is of interest to compare the radioactivity of the filter sample with that captured by the charcoal trap. The ^{131}I concentration in the charcoal trap (Fig. 1) in the 5–6 May sample was indeed between 3 and 4 times greater than that of the filter, suggesting that 65–70% of the radioiodine was present in gaseous form eight or nine days after injection, a value slightly less than that derived at Studsvik. In the succeeding 10 days, the ratio of ^{131}I in the trap to that in the filters decreased to ~ 2 , suggesting processes for converting gaseous to particulate iodine.

The level of ^{131}I observed in the surface air is of the same order of magnitude as that seen over Japan³ after the high-yield nuclear test by the Soviet Union in October 1961, which ranged from 0.02 pCi m^{-3} to 14 pCi m^{-3} (0.74 to 518 mBq m^{-3}).

The concentrations of radionuclides in rain water followed those observed in the atmosphere. During the sampling period, the frontal zone lay along the Japan islands, and the result of the cyclic passage of the pressure drop was that rainfall amounted to 110 mm between 1 and 17 May. The highest concentration of ^{131}I in rain water was found on 5–6 May (163 Bq per litre), after which the concentration decreased steadily. The maximum deposition of ^{131}I was, however, found on 6–7 May, because of the relatively large

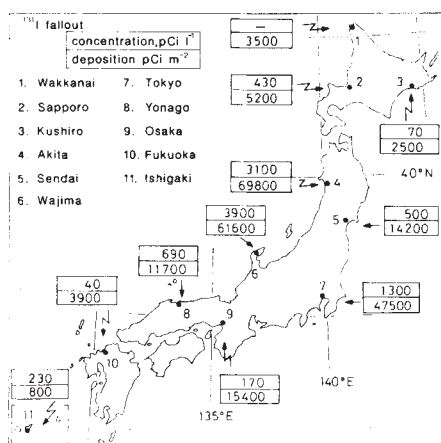


Fig. 2 The ¹³¹I concentration in rain water and the deposition at eleven stations in Japan during the period from 1 to 8 May 1986.

amount of precipitation then. It was confirmed chemically that 80% of the dissolved iodine was in the form of iodide, with the remainder as iodate.

The spatial distribution of ¹³¹I was determined by the surveillance network of the Japanese Meteorological Agency (Fig. 2). The largest amounts of ¹³¹I deposition were on Honshu and amounted to 60 mCi km⁻² (2,220 MBq km⁻²). Radioactive material was carried to this region of Japan by the Northern Hemisphere westerlies at a speed of ~1,000 km per day, and reached the middle part of Honshu in the west to south-west winds prevailing during this period.

Deposition of ¹³⁷Cs at Tokyo amounted to ~2 mCi km⁻² (0.074 MBq km⁻²) from 1 to 17 May, which is ~2% of the cumulative ¹³⁷Cs deposition since 1959 from nuclear tests¹.

Monitoring Minsk and Kiev students after Chernobyl

SIR—In the first few days following the incident at Chernobyl, very little information was available on the size of the release of radioactivity and the potential for further releases. However, the monitoring data from Scandinavia indicated a release significantly greater than that associated with the 1957 Windscale accident. As a consequence, the British Foreign and Commonwealth Office decided to recall British students close to the affected area, mainly from around Minsk and Kiev. The National Radiological Protection Board (NRPB) decided, after consultation with interested parties, to be present at Heathrow Airport, London, to carry out monitoring of the students.

NRPB stressed that the main purpose of the monitoring was to give reassurance to the students, and that we were expecting positive results in our measurements of radioiodine, but at a level that would give no cause for concern. We also hoped that our measurements would provide the first information of activity levels close to the

Our measurement of ⁸⁵Kr may be of particular interest because of the large proportions of this radionuclide among the fission products. Before the arrival of the high-level activity from the Chernobyl plant, the atmospheric concentration of ⁸⁵Kr was 0.93 Bq m⁻³, but increased to 1.04 Bq m⁻³ on 7 May, falling steadily to the normal concentration within a week. After the Three Mile Island reactor accident, ⁸⁵Kr was found only in the immediate vicinity; our detection of excess amounts of ⁸⁵Kr at a distance of 8,000 km from a reactor accident is the first measurement of this kind.

Further information on the accidental release of radioactivity will come from our investigation of alpha and beta emitters in the fallout. The temporal and spatial variation of the radioactivity should give valuable information about transport and scavenging processes in the atmosphere.

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incident area, so permitting some estimate of the severity of the release to be made. The portable radiation monitor for measuring radioiodine in thyroids was demonstrated with an ¹³¹I source containing a level of activity equivalent to that which, in a person's thyroid, would give the ICRP annual dose limit for a member of the public.

Travellers from Moscow had their thyroids checked and all were clear of radioiodine. The Minsk and Kiev students were all dressed in British Airways track-suits with their personal clothing held in polythene bags. It was explained to them that their thyroids would be checked for radioiodine and their clothing for radioactive contamination. When the radiation

thyroid monitor was demonstrated and the count rate corresponding to the ICRP annual dose limit indicated, we again stressed that we were not expecting to find this level of activity in their thyroids (see Table 1). The estimates of thyroid radioactivity are given as at the time of measurement, but the original intake of radioiodine has been calculated assuming all of it occurred the same day as the explosion in the reactor. For the Kiev students on the leeward side of the reactor at the time of the accident, this overestimates the initial intake.

In the seven volunteers for monitoring in the mobile laboratory, activity of ¹³¹I in the thyroid at the time of measurement varied between 1.9×10^3 and 6.4×10^3 Bq, in good agreement with the estimates of activity made using the hand-held monitors. The committed dose-equivalents to the thyroid were calculated to be in the range of 4 mSv to 14 mSv, or from 8% to 28% of the annual dose limit for the thyroid of members of the public; it was thus possible to tell all the students that the radioiodine content in their thyroids was low and gave no cause for concern.

The monitoring of the clothing for contamination inevitably was more time-consuming. Over 1,000 garments had to be carefully monitored with the monitoring probe in close contact with the surface of the clothing. NRPB staff used Nuclear Enterprise BP7 probes calibrated with a ⁹⁰Sr source to give 5 counts s⁻¹, corresponding to 1 Bq cm⁻². A decision had to be made on the acceptable level of contamination for the particular mixture of fission products concerned, which at that time was unknown. It was thought likely that a significant contribution would arise from the radioiodine and ¹³⁷Cs and that the critical exposure pathway would be irradiation of skin rather than ingestion. Using published NRPB information¹, an acceptance level of 30 Bq cm⁻² was adopted.

There remained the problem of determining the count rate for the contamination monitor corresponding to the 30 Bq cm⁻² for the "unknown" fission product mixture. The equivalent count rate for ⁹⁰Sr would have been 150 counts s⁻¹ and it was decided to reduce this level to 100 counts s⁻¹ because of the uncertainties in the exact constitution of the fission-product mixture. Measurements taken since the event have identified the constituents and an assessment of the count rate corres-

Table 1 Thyroid radioactivity of students returning to the United Kingdom

No. of persons	Count rate (counts s ⁻¹)	Approximate thyroid activity (Bq × 10 ³)	Calculated intake at 26 April 1986 (Bq × 10 ³)
1	85	6.9	53
1	70	5.6	43
9	50	4.0	30
11	40	3.2	25
33	30	2.4	18
34	20	1.2	9
10	10	0.8	6

ponding to 30 Bq cm⁻² indicates that 100 counts s⁻¹ is appropriate.

A more detailed assessment of the potential hazard presented by the contamination on the clothing, using the known fission products spectrum, has since confirmed that the critical exposure is indeed irradiation of the skin, not the possible ingestion of activity. The monitoring of clothing at Heathrow had established that garments were not extensively contaminated, but that there were isolated spots of contamination. At the clearance limit of 30 Bq cm⁻², an isolated spot of activity corresponding to 1.5×10^3 Bq would have passed the screening check for the clothing. This amount of activity if ingested would give rise to a dose of less than 1% of the annual dose limit for a member of the public.

The same spot of contamination on a person's skin would result in exposure to the skin in the immediate vicinity. The Ionising Radiation Regulations 1985, in discussing the assessment of dose from skin contamination, refers to the possibility of averaging the dose over an area appropriate to the circumstances, but in any event not greater than 100 cm². The students' contamination was on clothing, not on skin, and the attenuation of the beta radiation by the material, before reaching the skin, would be significant. In addition, the movement of the spot of contamination relative to a specific area of skin would result in lower levels of exposure. In the circumstances, an assumption of 100 cm² as an averaging area would seem appropriate. On this basis, the dose to the basal layer of the skin integrated to 12 hours after deposition would result in a dose of 0.5 mSv (or 1/10th the dose received from a dental X-ray to a comparable area of skin) and may be compared with the annual dose limit for a member of the public of 50 mSv.

Most of the clothing showed positive indications of contamination but only 2% of the clothing monitored was in excess of the "clearance" level. Contaminated clothing was put in polythene bags and has since been decontaminated at the Atomic Energy Research Establishment Harwell.

Scientists from Surrey University assisted in the clothing monitoring exercise and it was necessary to calibrate their instruments, which differed from NRPB's, to establish a count rate corresponding to the 30 Bq cm⁻² clearance level. The monitoring of the students' thyroids and their clothing took approximately 2 hours. The monitoring of the luggage and its contents still at that time in the aircraft clearly would have taken much longer and students were advised to leave these items.

The results of the monitoring enabled NRPB to reassure the students that their levels of dose were low and not a cause for concern. Bearing in mind, however, that

Minsk and Kiev are about 300 km and 100 km respectively in opposite directions from Chernobyl, the contamination levels on clothing demonstrate how severe the incident had been. The fact that both the thyroid monitoring and clothing monitoring results for the Kiev and Minsk students showed little variation indicated that the release must have continued for a number of days, during which time it was known that the wind had veered and blown in the direction of Kiev.

After the monitoring of the students, further checks were carried out on other travellers arriving from Eastern Europe. The results of thyroid and clothing contamination checks on a group of twelve employees from a British company working approximately 60 miles from Kiev were very similar to those experienced with the students. Other travellers from Warsaw showed positive but much lower levels of contamination and thyroid activity, while travellers from Moscow gave no detectable contamination. Monitoring facilities were made available to travellers returning from the affected parts of Eastern Europe via the NAIR arrangements², which are widely available throughout Britain to cover incidents involving radioactivity. This facility is still available to travellers returning from Eastern Europe but at this stage the justification is primarily the provision of reassurance to those members of the public who have need of it.

Monitoring has also been carried out at Dover of a group of travellers on a coach tour which had included a visit to Minsk, within a few days of the incident occurring, to Leningrad and Scandinavia. No thyroid or clothing contamination was detected.

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1. NRPB Publ. No. DL2 (1979).

2. Department of Health and Social Security Health Circ. HC(76)52.

Radiation levels in Eastern Europe

SIR—Immediately after news of the accident at Chernobyl became available on 28 April, the National Radiological Protection Board (NRPB) was asked by the UK Foreign and Commonwealth Office (FCO) for an appraisal of the radiation situation, on the basis of which advice would be given to embassy staff, residents in affected countries and prospective travellers.

The first Swedish measurements, external gamma dose rates and, later, concentrations of atmospheric and ground radioactivity, were used to estimate the

quantity of radioactivity released by means of NRPB's accident consequence code¹, MARC. Our first predictions of 29 April indicated a substantial release of the order of $10^{17} - 10^{18}$ Bq, which was expected to lead to tens of early radiation-induced deaths in the area around the site. On the basis of NRPB's recommended Emergency Reference Levels (ERLs)^{2,3}, it was thought that there would be a need for evacuation out to 20 or 30 km and for temporary food restrictions out to a few hundred kilometres from the site. FCO accordingly decided to evacuate students from Kiev and Minsk and to advise people not to travel to the western Soviet Union or to north-east Poland unless absolutely necessary.

Because some of the early data were contradictory or not reported in a recognizable way, it was concluded that radiation levels could also be obtained directly through the British embassies in various countries of eastern Europe. Embassies were accordingly equipped to measure external radiation dose rates from gamma rays by means of simple but sensitive instruments consisting either of the Mini Instruments 5.10 environmental exposure rate meter or a hybrid instrument using the same detector. All were calibrated before despatch. The British embassies at Moscow (Soviet Union), Warsaw (Poland), Bucharest (Romania), Budapest (Hungary) and Prague (Czechoslovakia) were the first to be sent instruments, followed after a few days by Sofia (Bulgaria) and Belgrade (Yugoslavia). The embassies were also asked to return samples of tap water, fresh milk, vegetables and grass.

Embassy staff measured gamma dose rates near their embassies and often at other locations (see Table 1). No allowance has been made for the natural background dose, normally between 0.1 and 0.2 µSv per hour. The measured dose rates quoted are the highest known to NRPB, but for some countries, where the first measurements were made about a week after the accident, the measured rates may be less than the peak rates. The estimates in Table 1 of the average external dose from deposited material that will be received in a year are based on the assumptions of a mix of radionuclides, allowing for radioactive decay and natural weathering⁴, that people spend 90% of their time indoors⁵ and that shielding by buildings reduces exposure by a factor of ten⁶. In all cases, about one third of the dose will be received within the first months, with the remainder delivered over the rest of the year at a fairly uniform rate. Our estimated doses should be compared with the external dose due to natural radiation, typically 0.6–0.7 mSv per year. The highest doses outside the Ukraine and Byelorussia are estimated for north-east Poland and Romania, but even

Table 1 External γ -ray doses

Location	Source of data	Highest measured dose-rate (1) ($\mu\text{Sv h}^{-1}$)	Representative present values on 14 May ($\mu\text{Sv h}^{-1}$)	Estimated total dose in a year (mSv)
Soviet Union				
Moscow	Brit. Embassy	0.2	0.1	—*
Kiev	Local scientist	30		†
	Official		2	2
Lithuania	Official	—	0.1	0.1
Chernobyl	Official	150	3	‡
Yugoslavia	Yug. authorities	1.6		0.3
	Brit. Embassy		0.4	0.3
Bulgaria	Official	0.7	0.5	0.3
	Brit. Embassy		0.25	0.2
Poland				
North-east	Official	4.5		0.8
	Brit. Embassy		0.8§	0.6
Warsaw	Brit. Embassy	0.4	0.2	0.1
Czechoslovakia				
Prague	Brit. Embassy	0.9	0.5	0.2
Hungary	Official	0.4	0.2	0.1
	Brit. Embassy		0.25	0.1
Romania	Brit. Embassy	3.6	0.9	1

Official figures are from governmental sources in the countries concerned.

* It is likely that dose-rates given here are measurements of fluctuations in natural background radiation in Moscow. However, if the higher reading is taken to indicate the presence of radionuclides from the Chernobyl reactor then the estimated dose over the year is less than 1 mSv.

† If this dose-rate is representative, it suggests a dose over the year of 20 mSv.

‡ It is not clear where in the Chernobyl region these dose-rates were measured. The dose-rates are likely to vary considerably with distance from the reactor so it is unreasonable to estimate a dose at Chernobyl.

§ Measured on 10 May. Earlier measurements indicate that dose-rates in northeastern Poland are falling and so it is likely that the dose-rate here on 14 May is $< 0.8 \mu\text{Sv h}^{-1}$.

these are only a fraction of the recommended annual dose limit for members of the public of 5 mSv per year.

The most significant radionuclides in the samples of food from the Soviet Union, Poland, Hungary, Czechoslovakia and Romania measured by NRPB are ^{131}I

Table 2 ^{131}I concentrations in milk

Location	Source of data	Measured concentration (Bq l^{-1})	Date milk obtained
Soviet Union			
Moscow	NRPB	35	3 May
Moscow	NRPB	18	7 May
Yugoslavia	Official	50–150	early May
Poland			
General	Official	30–2,000	29–30 April
General	Official	20–1,000	1–2 May
General	Official	30–600	3 May
Warsaw	NRPB	210	5 May
Czechoslovakia			
Prague	NRPB *	110	8 May
General	Official	100–500	8 May
Hungary			
Fresh milk	Official	100–2,600	1–4 May
Fresh milk	Official	50–1,200	5–9 May
Fresh milk	Official	< 600	11 May
Supermarket	NRPB	16	5 May
Romania			
Local market	NRPB	420	4 May

* Polish government source, not Czechoslovakian.

and ^{137}Cs ; levels in tap water were all low, in the range 1–100 Bq per litre of ^{131}I . In milk (Table 2), levels of ^{131}I peaked at the end of April or in early May, and have since fallen. The wide variation of measured ^{131}I probably reflects the rainfall pattern and the use of fodder other than grass for some cattle, but the highest levels reported, from Poland and Hungary, are comparable with the Derived Emergency Reference Level (DERL)³ for the peak concentration of ^{131}I in milk of 2,000 Bq per litre; in both countries, restrictions of milk supplies from cattle grazing pastures were introduced. Concentrations of ^{131}I in milk samples obtained by embassy staff were, however, all well below the DERL and are at present similar to those measured for the northern part of the United Kingdom⁷.

There is a wide variation of the concentrations of ^{131}I and ^{137}Cs (not measured for all samples) in vegetables measured by national authorities and by NRPB, possibly because of the rainfall pattern, the circumstance that some had been grown under cover and the method in which they had been prepared; samples analysed by NRPB show that concentrations in leafy green vegetables can be substantially reduced by the removal of outer leaves, washing and kitchen preparation in general.

Shortly after the Chernobyl accident,

FCO advised the avoidance of fresh milk and free-range eggs, the avoidance of surface-grown vegetables and, in any event, washing and peeling (as for fresh fruit) in the Western Ukraine, Byelorussia, Moscow, Lithuania and northeastern Poland. This list was later modified to exclude Moscow and to include Romania. As more evidence becomes available, it should be possible to modify this advice further, although it is likely that precautions will be required for some time in the Western Ukraine and Byelorussia. Other foods, notably meat, from these areas need to be kept under review.

The early appraisal of the situation, based on an estimate of the magnitude of the release, was sufficient to give a reasonable general picture, but far more information is required to give specific advice for individual cities, regions and countries. The data obtained by British embassy staffs, to whom we are grateful, has been invaluable. The situation in Eastern Europe remains under review.

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Living with nature

George Peterken

The History of the Countryside: The Full Fascinating Story of Britain's Landscape.

By Oliver Rackham. *Dent/Biblio, Totowa, New Jersey: 1986. Pp.445. £16.95, \$35.*

Countryside Conflicts: The Politics of Farming, Forestry and Conservation.

By Philip Lowe, Graham Cox, Malcolm MacEwan, Tim O'Riordan, Michael Winter. *Gower/Maurice Temple Smith: 1986. Pp.378. Hbk £19.50, \$35; pbk £8.95, \$16.*

TO ONE who came into countryside conservation in the 1960s, one of the more intriguing developments has been the subsequent demotion of science as an objective. As the authors of *Countryside Conflicts* point out, in Britain ecologists assumed the leadership of the nature conservation movement in the 1940s as part of the British Ecological Society's drive for government recognition. Science became the principal aim of nature conservation — we still call the important sites "Sites of Special Scientific Interest" — and the means by which the behaviour of species and of ecosystems were investigated. With nicely unscientific circularity we reserved sites for scientific research, and conducted scientific research with which the better to reserve and manage these sites. These days we stress other attributes and benefits, such as the seemingly nebulous values of heritage and wilderness, and seek to understand ecosystems more by their history and less by their mechanics. We recognize that science can only be a partial answer even to nature reserve management: even if we could afford the research needed to understand the needs of all species, we would still require other bases for deciding which species to favour.

Both of the books under review testify to this change, but in vastly different ways. Oliver Rackham, already famous as the author of three books on the ecology and history of woodlands, here expands into the countryside at large, though woodlands remain a major theme. Concentrating on the relatively natural elements, his aim is to show how the countryside came to assume its present character. Unlike analogous, but far less detailed works on the making of the English landscape, which apportion history into broad eras then describe the most notable developments within each, Rackham takes each individual element in the landscape separately. Thus, we have chapters on woodland, wood-pasture, fields, hedges and

field walls, highways, heath-land, grass-land, ponds, dells and pits, and many other features. These chapters follow others on landscape regions, and the use of historical evidence. There is also a short chapter on conservation, and brief conservation sections in other chapters.

Rackham's numerous admirers will once again be delighted with his lucid, precise and non-technical style and illustrations, and his entertaining presentation. Drawing copiously on original historical records, he fascinates the reader with the human interest that runs through the story and with the detail that has survived the centuries. In what other ecological textbook do we learn, for example, that "for his Christmas dinner, 1251, Henry III had 430 red deer, 200 wild swine, 1300 hares... and lampreys without number" as some indication of the pro-

duce of wood-pasture? The book also contains important original research, such as the detailed analysis of the ecological content of Anglo-Saxon charters. Numerous myths are demolished, notably the notion that woods, heaths, fens and so on are normally destroyed by use of the native vegetation, when in fact agriculture and plantation forestry have been the main culprits. Throughout, this is essentially a personal statement, which draws its strength from the author's exceptional ability to perceive and express patterns in a mass of detail. Other people's work is used, not to provide syntheses and concepts, but to provide good examples, amplify details, and partially correct Rackham's geographical bias to East Anglia, south-west England and south Wales.

Whereas Rackham covers the full span of countryside history from an ecological standpoint, the authors of *Countryside Conflicts* deal with the recent history of the political, social and economic side of conservation. After reviewing the twentieth-century rural preservation movement, the post-1945 planning system, and the political and economic background to forestry and agriculture over the past 40 years, Lowe and his colleagues are almost wholly concerned with the 1970s and 1980s. They analyse the two "opposing forces" in the countryside — the farming, land-owning and timber-growing "lobbies", and the conservation "movement" (this verbal loading is characteristic) — then describe in some detail the manoeuvrings behind the Wildlife and Countryside Act of 1981 and its subsequent implementation. Then, in what for many will be the most interesting section, they describe the well-known conflicts with farming in Exmoor, West Sedgemoor and the Halvergate Marshes, and with afforestation on the Berwyn Mountains. The final part is a review of needed reforms, more a manifesto than a practical guide, including proposals to merge the Nature Conservancy Council with the Countryside Commissions, and to reform countryside designations such as "Area of Outstanding Natural Beauty".

Rackham's publishers claim that his book is one of the most important on the countryside to appear in recent years, and for once the hype may be right. Certainly, his earlier writings have so fired the interest and imagination that both Parliament and the Forestry Commission dance to his tune, at least in so far as broadleaf woods are

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

In the wild — Llanberis Pass, in Snowdonia National Park. The picture is reproduced from Wildest Britain: A Visitor's Guide to the National Parks, by Roland Smith with photographs by Mike Williams. Publisher is Blandford, price is pbk £6.95, \$12.95.

concerned. *Countryside Conflicts*, too, clearly seeks to influence the course of rural events. Where, then, would the two books lead us?

Countryside Conflicts takes the view that conservation should be an operative principle of all land management, not a separate element within reserves and designated sites. This requires a change in direction and purpose of forestry and agriculture. Much of the authors' writing implies a conservative view of the landscape: they seek mechanisms to maintain hill farming and thereby keep the landscape we have. To make this possible they nevertheless show some willingness to sacrifice the wild, for example by advocating grants which would support initial operations such as draining, fencing and planting. They are against conifer afforestation in the uplands, but support broadleaf afforestation in the lowlands, only hinting at how they would avoid setting this woodland on surviving fragments of semi-natural habitat. Indeed, throughout they are vague about what should physically be done on the land.

There is little doubt about Rackham's sense of value. He appreciates the antique features, which have "meaning", particularly the natural elements and time-honoured associations between man and nature. His attitude is exemplified by the claim that "ten thousand oaks of 100 years old are not a substitute for one 500-year-old oak", with the implication that they are less valuable. Inevitably he is scathing about the effects of modern agriculture — "fertility comes in a sack" — and considers not only that it should not be extended but that it should retrench towards more conservative forms of land management.

This is all very well. Many people, including myself, understand these very points and sympathize with his attitudes. Experience has taught us, however, that many others value a single 100-year-old oak above its 500-year-old grandparent, both as timber and scenery. Such people see beauty in order, cultivation and production, and rejoice in increased productivity of farmland and plantations. Others again seek refreshment and beauty in wilderness, and would minimize the effects of man. Rackham sees variety, significance (the latter-day "scientific interest"), permanence and security in traditional conservative use of resources, and thereby rejects both views: it is valuable to have his corner so cogently argued.

Vague though they are on the practicalities, these books converge in their antagonism to much of modern agriculture and forestry, and their support of the semi-wilderness maintained by traditional practices. Living with nature is the modern theme of conservation. □

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The need to know about lipids

Howard Goldfine

Biochemistry of Lipids and Membranes.

Edited by Dennis E. Vance and Jean E. Vance. *Benjamin/Cummings*:1985. Pp.593. \$34.95, £34.95.

INTEREST in lipids among biochemists and molecular biologists appears to go through cycles ranging from high enthusiasm to near neglect. Recently, a decided upturn in awareness can be detected. Although the phosphatidylinositol cycle has been studied for 30 years, its physiological significance has only recently come to wider notice. Adding to this increase in attention being paid to lipids have been the ripple effects of discoveries on the arachidonate cascade, the regulation of cholesterol biosynthesis and the molecular biology of the low-density lipoprotein receptor, and the finding that the platelet-activating factor is an ether lipid analogue of phosphatidylcholine.

The appearance of a substantial volume devoted to the biochemistry of lipids and membranes will be welcomed not only by those who have long engaged in lipid research, but also by those who have been drawn to it by following leads in such areas as viral tumorigenesis and hormonal regulation, or by a desire to understand how membranes are assembled and how they work. The editors set themselves a double goal — to produce a text for use in advanced biochemistry courses devoted to lipid and membrane biochemistry, and a reference volume for basic and clinical researchers. They have largely succeeded in meeting the second objective by having each of the 15 chapters written by knowledgeable and active specialists. The information is up to date and the subjects are presented in considerable depth, backed up by references to recent reviews and primary publications. As might be expected in a multi-authored work, some contributors show a tendency to advance their own views, but the balance is generally reasonable.

While the need for a text at this level may not extend to many graduate programmes in the biomedical sciences, where such courses exist or are contemplated this volume will prove very useful for both students and their teachers. Most of the chapters begin with a brief historical essay touching on key developments and figures in the field concerned, and end with a discussion of future directions and areas in need of further development. In addition to chapters on traditional areas of lipid metabolism, there are helpful articles on the assembly of membrane lipids and proteins, and a good but somewhat

abbreviated discussion of the physical properties and functional role of lipids in membranes.

A recurrent theme in the book is the paucity of information (with a few notable exceptions) that we have on the regulation of lipid metabolism, either at the level of the gene or by modulation of enzyme activities. Students looking for research problems in cell biology will readily find them here. The tools for solving such problems are becoming more sophisticated through the development of methods for solubilizing and purifying membrane proteins, the production of monoclonal antibodies, and the ability to isolate and clone the genes for the proteins of lipid metabolism. Answers may not come easily but, as this volume makes clear, the search should be both stimulating and rewarding. □

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Dishing the dirt

Richard P. Wayne

Atmospheric Chemistry and Physics of Air Pollution.

By John H. Seinfeld. *Wiley*: 1986. Pp.738. \$59.95, £57.45.

JOHN Seinfeld defines air pollution as "any atmospheric condition in which substances are present at concentrations high enough . . . to produce a measurable effect on man, animals, vegetation, or materials". Dusts, pollen, smoke and volcanic gases and ash can undoubtedly exert deleterious effects, although they are part of the "natural" atmosphere. Mankind has, however, discovered all manner of means of further polluting the atmosphere on scales ranging from the local to the truly global. Seinfeld's hope is that the scientific study of pollution will provide pointers to finding the optimum way of abating the problem in its manifold forms.

Research into air pollution encompasses several disciplines that are only loosely connected, and Seinfeld makes a determined effort to show how the different facets relate to each other. He first considers the sources of pollution, and the effects that various pollutants have on atmospheric properties, on materials, on vegetation and, above all, on human health. Often, the substances released as primary pollutants are not themselves the agents of damage. Rather, chemical transformations are responsible for the formation of a secondary species that is harmful. Such transformations range from the production of acids from the oxides of nitrogen and sulphur accompanying the com-

bustion of fossil fuels, to complex catalytic cycles in the stratospheric ozone layer that could alter the Earth's protection from solar ultraviolet radiation. Seinfeld has to consider, therefore, atmospheric chemical processes occurring both in the gas phase and within atmospheric liquid water, and he notes that several mass transfer steps must precede such aqueous reaction.

Aerosols, which are suspended particles of solid or liquid, play an important part in pollution studies. They degrade visibility, and can also act as condensation nuclei for the growth of larger liquid droplets. Seinfeld has chosen to present quite a detailed treatment of aerosol behaviour, offering a general review of aerosol physics, with emphasis on the dynamics of individual particles and of populations, and the thermodynamics of aerosols and of nucleation.

The dynamics of the atmosphere itself determine the dispersal and transport of pollutants. Logically, then, discussion of pollution meteorology occupies the next part of the book, and a further main section deals with atmospheric diffusion.

Seinfeld has achieved his objective of providing a comprehensive treatment of pollution processes. He suggests that the work could serve as a textbook for a course on the atmospheric aspects of air pollution, but readers would have to be pretty well equipped with a knowledge of applied mathematics to cope with the second half of the book. Indeed, I have slight reservations about the amount of quantitative detail presented here; the physics is largely relevant to idealized situations, and the implied precision is strangely at variance with the level of our understanding of the sources, effects and chemistry of pollutants.

The final chapter is devoted to a discussion of acid rain, and Seinfeld shows how the topic serves as an elegant summarizing problem which brings in almost all of the features discussed earlier in the book. In many ways, this section would be a good starting point for a beginner. For specialists, this is a most useful reference book, while for those with traditional scientific or engineering backgrounds it will prove a rigorous and trustworthy guide to a complicated subject. □

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• Also published recently by Wiley is *Air Pollutants and Their Effects on the Terrestrial Ecosystem*, edited by Allan H. Legge and Sagar V. Krupa, which is said to be "the first integrated work to present all current research on the effects of pollutants on vegetation, soils, and ecosystems". The book is Vol. 18 in the series *Advances in Environmental Science and Technology*; price is \$80, £82.75.

Faith and the ways of science

Robert John Russell

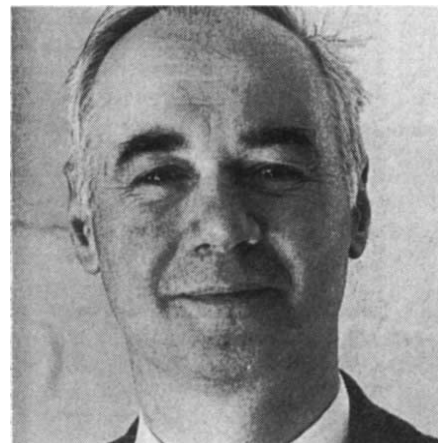
One World: The Interaction of Science and Theology. By John Polkinghorne. SPCK, London/Fortress Press, Philadelphia: 1986. Pp.114. Pbk £4.50, \$7.50.

JOHN Polkinghorne is Honorary Professor of Theoretical Physics in the University of Kent, a Fellow of Trinity College, Cambridge, and the Vicar of Blean, Kent. In his brilliant and artful exposition of contemporary physics (*The Particle Play*, published by W.H. Freeman in 1979), Polkinghorne acknowledged his identity as a "Christian believer", gave his judgement that the world-views of Christianity and science can be in "consonance" and announced that he was leaving his position as Professor of Mathematical Physics to train for the Anglican priesthood. *One World* is not the first of Polkinghorne's books to examine the relationship between religion and science. Appearing in 1983, *The Way the World Is* was a consequence of his "coffee shop" discussions about Christianity with his fellow scientists. Now, in this new book, we see his earlier ideas taking root in the context of contemporary philosophy and theology, though with the same distinctive style, clarity of thought and dry passion which his admirers (myself among them) have come to expect and appreciate.

In *One World* Polkinghorne defends the thesis that science and theology are "capable of mutual interaction" because they both explore "aspects of reality". He espouses a critical realist philosophy and argues against reductionism by the following epistemological claim: that the various fields of study, such as psychology or biology, form a hierarchy in which each field is relatively autonomous from the others. He sets the stage for his case by describing how contemporary physics has overturned the Enlightenment view of the objective and determinate status of the world, and how contemporary philosophy has exposed earlier, simplistic interpretations of the scientific method. Twentieth-century theology, too, has moved beyond dogmatics to a critical analysis of religious experience and so "theology, like science, is corrigible". Hence theologians should seek coherence, economy, adequacy and existential relevance.

Writing with precision and elegance, Polkinghorne captures the majesty and mystery of our current view of nature: elusive (unpicturable but real) and yet intelligible (through mathematics); problematic (for example, the conflicting interpretations of quantum theory); contingent yet lawful; tight-knit (human evolution in

an "anthropic" cosmos) and yet futile (given the cosmological scenarios for a lifeless distant future); complete ("the one God who is... truly dead is the God of the Gaps") and incomplete ("There is more to the world than physics can ever express"). Hence Polkinghorne urges that theology and science can intersect without conflict. Regarding origins, theology can see God as the ground of reality rather than a cause among causes, thus allowing Polkinghorne to affirm "God and the big bang". Chance, too, is not necessarily a sign of God's absence. Instead "both the lawful necessity of the world and the role that contingent chance has to play within it are aspects of [God's] great creative act". Polkinghorne concludes that "we live in



John Polkinghorne — "There is more to the world than physics can ever express".

one world and science and theology explore different aspects of it", a world whose "multi-layered unity" is grounded in God.

This is a satisfying book, though it has some problems. Polkinghorne frequently fails to cite the people whose ideas he seems particularly indebted to (notably Ian Barbour and Arthur Peacocke). More to the point, he does not adequately show how the *contents* of science (and not just its epistemology or methodology) should affect constructive theology and thus how these fields actually address "one world". Still, *One World* offers a balanced, though almost precariously brief, version of the challenges and promise of science and theology. Much to his credit, Polkinghorne never pleads his case, preaches to the reader or draws on fringe arguments, but his personal convictions are clear throughout. As a candid and compelling statement of faith given by an internationally distinguished scientist — now priest and theologian — I welcome Polkinghorne's new book and urge him on in the journey we share. □

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Sound of erudition

James Cullen

Science and Civilisation in China, Vol. 6: Part 1. Biology and Biological Technology: Botany. By Joseph Needham. Cambridge University Press: 1986. Pp. 718. £50, \$95.

DR NEEDHAM'S series *Science and Civilisation in China*, which he began some 30 years ago, needs little introduction here. This latest volume is by Needham himself, with the collaboration of Li Gwei Djen (Cambridge) and Huang Hsing-Tsung (Washington). Part 2 of Vol. 6, *Agriculture*, by Francesca Bray, was published in 1984, and a further part, continuing the subject of botany, is being prepared by Georges Métaillé of the Musée d'Histoire Naturelle in Paris.

In covering the history of botany in China from its remote origins to the impact on it of Western science in the late nineteenth century, Needham has surveyed a huge amount of literature. There are three bibliographies — of Chinese and Japanese books before 1800; the same since 1800; and books and articles in Western languages — which altogether occupy 99 pages. This vast quantity of source material is processed with a breathtaking display of erudition.

Throughout, Needham contrasts what was happening botanically in China with corresponding events in the West, and stresses two important differences: first, that the history of botany in China, like that of many of the physical sciences, is more continuous than in the West — in China there were difficult times, but no aching void equivalent to the Dark Ages; second, following the rapid advance of the scientific method in the West, Western botany overtook Chinese in both the pace and scope of its development. Needham categorizes Chinese botanical taxonomy (for the volume is, by necessity, mainly a history of taxonomy) as having reached a "Magnolian" or "Tournefortian" rather than a "Linnaean" or "post-Linnaean" level.

After the introduction, the volume is divided into five large sections. The first, "The Setting: China's Plant Geography", is a brief survey of the floristic divisions of the country as recognized by botanists today, together with a discussion of the development of Chinese ideas on the topic in which the stress is on soils and soil types.

The second section, "Botanical Linguistics", covers Chinese terminology for plant description and nomenclature. This fascinating if complex subject is clearly presented for those who know little about ideographic writing. Needham concludes it with some thoughts on the appropriateness of ideographs for taxonomic purposes. Because they are made up of superimposed radicals, ideographs can convey classificatory ideas very satisfactorily ("it might well be said that a language the dictionaries of which have to be based on taxonomic principles is a language remarkably congruent with the nature of biology" — p. 178); indeed, Needham compares them with various coding systems (some of them machine-readable) developed in recent years.

"The Literature and its Contents" follows and is the heart of the volume. A wide variety of texts is surveyed for their botanical content, including lexicographic and encyclopaedic works, medical books, books on wild (emergency) food plants, monographs and tractates, and works on historical and exotic botany. This section is too densely packed to describe in any detail, but one of its most interesting topics is that of the monographs: some little knowledge of these has filtered through to Western botanists, but their age-range and scope are not so well known. Monographs on citrus fruits (*Citrus*, *Fortunella*), bamboos (*Gramineae Bambusoideae*), peonies (*Paeonia*) and chrysanthemum, among others, are discussed in some detail; the amount of study devoted by the Chinese to cultivated varieties is noteworthy.

The final section deals with a less narrowly taxonomic subject, that of pest

control among cultivated plants, and in particular the use of biological control in agriculture. Entitled "Plants and Insects in Man's Service", it is by Huang Hsing-Tsung and contains much of interest, especially the account of the use of a species of ant (*Oecophylla smaragdina*) to control pests in orange orchards, with the use of bamboo bridges from tree to tree to "facilitate migration". This means of control appears to have been in consistent use in South China from AD 300 to 1960. Its effectiveness is, however, doubted by

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Aquatic frog (wa) — detail of an illustration in Paul U. Unschuld's Medicine in China: A History of Pharmaceutics, newly published by University of California Press. Price is \$47.50, £40.50.

some authorities, both Chinese and Western.

The whole book is a pleasure to read: informative, stimulating and written with a wit and passion that are found more often in historical than botanical texts. Needham expects quite a lot of his readers: on p. 373, quoting a long passage from Han Yen-Chih's *Chü Lu*, the footnote to part of it reads:

The sentences in inverted commas were, as Pelliot recognised, borrowed by Han Yen-Chih from a 'Letter accompanying a Present of Oranges' written by Liu Hsun.... He worked them in, as they were appropriate, just as we might use a few words of Sir Thomas Browne, knowing that all educated readers would respond to the echo.

How many botanists might so respond? However few, the book contains so much valuable "sound" that the loss of a few echoes is not significant. □

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Experimental forecasts of El Niño

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Experimental forecasts of El Niño events occurring since 1970, made with a deterministic model of the coupled ocean-atmosphere system, indicate that El Niño is generally predictable one or two years ahead. A forecast for 1986 is also presented.

THE devastating climatic events of 1982–83 were the most recent extreme phase of the irregular cycle of atmospheric and oceanographic changes known collectively as El Niño and the Southern Oscillation (ENSO)^{1–5}. If forecasts could provide sufficient warning of an impending episode, appropriate planning could reduce human suffering and economic loss^{6,7}. In 1982 the state of information gathering and forecasting activities was clearly inadequate: the event was well under way before it was recognized. Statistical procedures reported more recently^{8,9} could have forecast it in the spring of 1982, a few months in advance of the peak warming.

Forecasts of El Niño at longer lead times could be impossible in principle. Perhaps the intrinsic behaviour of the coupled ocean-atmosphere system is such that states which initially differ by a small amount diverge very rapidly. If so, it would be impossible to observe initial conditions with sufficient precision to determine the subsequent evolution over many months. Even if skilful forecasts at longer range are possible in principle, they may be unattainable in practice because of inadequate models or the limitations of available data.

While it leaves many questions unanswered, the work reported here offers grounds for optimism. We have used a deterministic numerical model of the coupled ocean and atmosphere in the tropical Pacific region to make forecasts of all the El Niño events since 1970. The results indicate that the large-scale warmings associated with these events could have been predicted a year or more in advance.

The numerical forecasting model^{10,11} is a coupled model for the evolution of the ocean and atmosphere in the tropical Pacific region. Only deviations from the mean are calculated explicitly; mean conditions are specified from monthly climatological data. No statistical procedures are used in the forecasts. Model variables evolve deterministically, according to the physical laws governing the atmosphere and oceans. The model was originally developed for abstract studies of large-scale ocean-atmosphere interactions in the tropics and is far simpler than the numerical models used operationally for weather prediction. Nevertheless, it reproduces much of the characteristic spatial and temporal structure of observed El Niño events, including their recurrence at irregular intervals with an average period of 3–4 yr (refs 10, 11).

In part, the simplifications were designed to serve a didactic purpose: insofar as the model simulations of ENSO are judged to be correct, one may conclude that the omitted processes are not essential for the existence of the ENSO cycle. When the model is to be used for forecasting, however, verisimilitude is clearly desirable and the simplifications are a handicap. The model's predictive skill is probably decreased by the absence of the 30–60-day waves so prevalent in the tropics¹², as well as by the lack of any variability generated in mid-latitudes. The model exhibits too little variability in the western equatorial Pacific and overstates easterly wind anomalies in the east. It cannot reproduce episodes of strong cold anomalies. All of these flaws (and others) reduce the realism of its ENSO simulations.

On the basis of model results, we have suggested a theory for the ENSO cycle^{10,11} along lines proposed by Bjerknes¹³ almost two decades ago. Many investigators since Bjerknes have substantially advanced our understanding of the tropical atmos-

phere and oceans, allowing his original framework to be bolstered and elaborated. Our theory and model design draw on this collective progress^{1,14}, only a brief sketch of which is possible here.

Ocean-atmosphere interaction

Work on equatorial ocean dynamics following the pioneering sea-level studies of Wyrtki^{15,16} established that changes in the eastern equatorial Pacific could be caused by remote wind changes to the west, with the signal propagating through the ocean along the equatorial wave guide¹⁷. Others explored the ways in which ocean dynamics^{13,18–20}, not surface heating anomalies^{4,13,21}, accounted for observed anomalies in sea surface temperature (SST). Experiments with atmospheric general circulation models^{22–24} showed that tropical Pacific SST anomalies could cause the meteorological changes characteristic of ENSO; other studies showed that simple models could largely account for the tropical aspect of the anomaly pattern^{4,25–28}.

If the winds alter the SST pattern, which alters the winds, then a coupled ocean-atmosphere model is clearly required. Although highly idealized, the models described in the literature^{29–33} provide considerable insight into the mechanics of growing and oscillating modes of the coupled system. (This work has been reviewed by McCreary³⁴).

In our model, the atmosphere and ocean both have an active role in ENSO: it is an oscillation of the coupled system. Its consequences are global, but the interactions vital to its existence all occur in the tropical Pacific region.

In the normal state, the tropical Pacific is warmer in the west and colder in the east; a centre of tropical rainfall overlies the pool of very warm water in the far western Pacific. The easterly trade winds blowing towards this atmospheric heating region drive warm surface waters to the west, while drawing colder sub-surface waters upward at the east. Thus, the temperature contrast responsible for the atmospheric circulation is maintained by that circulation—a positive feedback.

If the eastern ocean becomes warmer, then the rainfall spreads eastward with the warm water, and the surface winds slacken. As a result, some of the warm western water moves eastward and less cold water is drawn upwards; hence, the east becomes warmer still. Again, there is a positive feedback, leading to more westerly winds, warmer SSTs and a deeper thermocline in the east. This is an El Niño event.

Bjerknes¹³ discussed both of these 'chain reactions' but could not explain why there were transitions from one phase to the other in an endless cycle. The key idea in our theory requires going beyond the vertical plane along the Equator and considering the north-south circulation in the ocean^{4,18,29}. Approaching the peak of an El Niño event, water moves not only from west to east, but also poleward³⁵, emptying the reservoir of warm water at the Equator.

In the aftermath of the event, the heat loss results in colder than normal eastern Pacific SSTs and, consequently, stronger than normal easterlies—an enhanced version of the non-El Niño state. The transition back to the El Niño state cannot take place until enough warm water flows back from higher latitudes to refill the equatorial heat reservoir. Episodes of weak easterlies are frequent, but until the reservoir is refilled there is not enough

warm water available to sustain the 'chain reaction' that generates an El Niño event.

Our focus on the heat content of the equatorial band arose from theoretical considerations and numerical model results. There is also observational evidence supporting these ideas. On the basis of tide gauge data, Wyrtki³⁶ has independently advanced a similar hypothesis.

The time it takes to restore the equatorial heat content depends on the strength of the coupling between atmosphere and ocean¹⁰. The phenomenological 'coupling strength' comprises a number of factors, such as the sharpness of the thermocline, advective speeds, oceanic temperature gradients, dissipation rates, oceanic wave speeds and the sensitivity of the surface wind to SST contrasts. All of these vary seasonally and spatially, in addition to changing with the state of the ocean-atmosphere system. In addition, the flow of warm water back to the Equator is affected by higher-frequency events such as the 30–60-day waves¹² in the western Pacific.

The irregularity of the interval between El Niño events reflects the variability of this refill time; it is this variability which makes the prediction of El Niño difficult. It is not known whether it is more a result of high-frequency noise³⁷ or of the nonlinearities intrinsic to the ENSO cycle³⁸. Although either implies that the ENSO cycle cannot be predicted arbitrarily far ahead, neither precludes prediction at useful lead times.

The ENSO scenario outlined here has further implications for the prospects of forecasting an El Niño event. As the essential interactions take place in the tropical Pacific, data from that region alone should be sufficient for forecasting. Also, our model implies that the future evolution of the ENSO cycle could not be predicted without knowledge of the upper-ocean heat content. The radiative relaxation time of the atmosphere is ~1 month, and the thermal damping time of the ocean surface layer is at most a few months (4 months in the model). Evidence that El Niño can be predicted at significantly longer lead times implies an active role for the sub-surface ocean, which has substantially greater thermal inertia.

In the model, the sub-surface thermal structure reduces to a single variable, the anomaly in the heat content of the upper ocean, or, equivalently, the thermocline depth anomaly. (A recent empirical study highlights the same variable³⁹.) The analysis above implies that this variable is an essential initial input for forecasting. As available observations are insufficient to specify a complete field of thermocline depth anomalies, the following procedure was adopted. A field of monthly mean surface wind stress anomalies was derived from ship observations over the tropical Pacific⁴⁰; a 1–2–1 filter in time, longitude and latitude was then applied to the winds analysed. The anomalies used are the deviations from the average of the same calendar month over the previous 4 years; for example, the May 1985 anomaly is the deviation from the mean of May 1982, 1983, 1984 and 1985.

The period up to the forecast initial time was simulated by forcing the ocean component of the coupled model with wind-stress anomaly fields specified for each month starting with January 1964. The computed SST anomalies were then used to run the atmospheric component of the model. The calculated anomalies in thermocline depth, currents, SST and surface winds were then used as initial conditions for a forecast with the coupled model. Note that the initial SST and wind fields are calculated to be compatible with the initial thermocline displacements within the model framework; they need not agree with observed SST or wind fields. The coupled model runs evolve from the initial conditions as in a true forecast model: no new data are introduced after the initial time.

Forecast results

Figure 1 compares a map of observed SST anomalies during the peak period, January 1983, with that from the forecast initiated in January 1981. The result is typical of the more

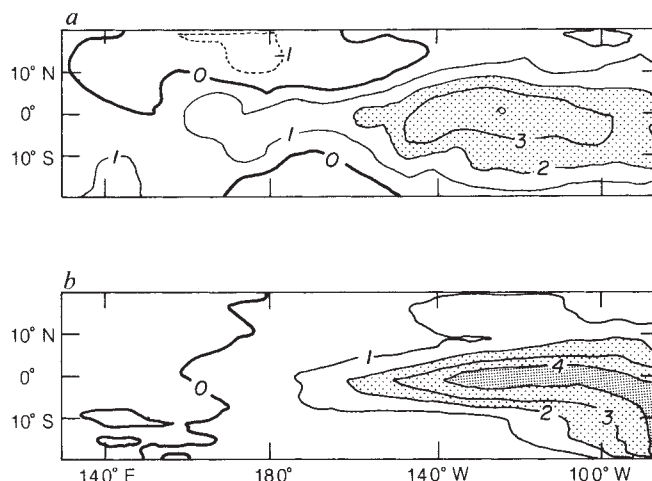


Fig. 1 Sea surface temperature (SST) anomalies (°C) in January 1983. *a*, Observed, based on the analysis of the Climate Analysis Center (CAC) of NOAA. *b*, Predicted by the model forecast initiated in January 1981, 2 years earlier.

successful forecasts. As in earlier work¹⁰, including cases with the same ocean model driven by observed winds²⁰, the gross character of the eastern Pacific El Niño SST anomaly is reproduced, although its meridional and westward extent is underestimated.

Forecasts were initiated in the periods preceding each of the El Niño events that have occurred since 1970; that is, the events of 1972, 1976 and 1982. An additional set was made for the 'non-event' of 1979. No forecasts were attempted in the years before 1970 because the wind analyses available for these earlier years are of distinctly lower quality. In each period there are six forecasts spaced 3 months apart, with the sequence ending in January of the nominal year. For example, for 1972 forecasts were initiated from October 1970, January, April, July and October, 1971 and January 1972.

The forecast results are summarized in terms of the SST anomaly averaged over the eastern equatorial Pacific area called NINO3 (5° S to 5° N; 90° W to 150° W). The NINO3 index was devised by the Climate Analysis Center of NOAA (National Oceanic and Atmospheric Administration) because a warming in this region strongly influences the global atmosphere². It is probably the best single indicator of an ENSO episode likely to affect global climate.

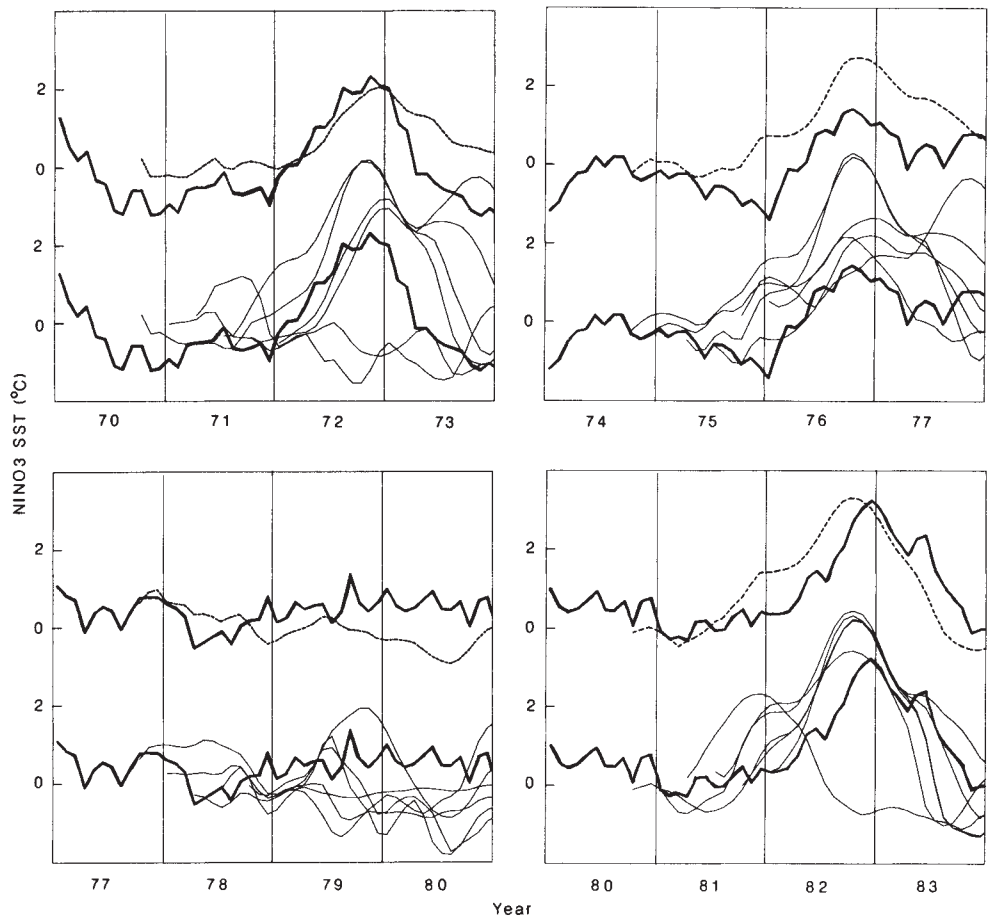
Figure 2 shows that the model generally succeeds in predicting warmings in those years when they were observed to occur, and

Table 1 Summary of forecast results for past years

Start month	No. right	No. of cases	Chance by coin flips
1-yr forecasts			
October	7	8	0.04
January	8	8	0.004
April	1	4	0.94
July	4	4	0.06
Total	20	24	0.0008
2-yr forecasts			
October	3	4	0.31
January	3	4	0.31
Total	6	8	0.14

Results correspond to the 24 forecasts of Fig. 2. For January, '1-yr forecasts' refers to the same calendar year; for all other start months, to the next calendar year. The last column shows the probability of correctly predicting at least as many cases as the model did by flipping a coin.

Fig. 2 SST anomalies ($^{\circ}\text{C}$) averaged over the eastern equatorial Pacific NINO3 region (90°W to 150°W , 5°S to 5°N) for four periods centred on 1972, 1976, 1979 and 1982. Heavy curves, observed values as reported by CAC/NOAA (note repetition of vertical scale). Light curves, values from six forecasts initiated at 3-month intervals from the October two years ahead to the January of the nominal year. Dashed curve, average of the six forecasts (the consensus forecast).



in predicting their absence when they did not occur. As with the observed events, the peaks in the NINO3 index are always forecast to take place at the end of the calendar year. The individual forecasts tend to give too strong an event, especially for the 1976 El Niño. The rise in temperature during 1976 is well predicted, but five of the six forecasts begin the year at least 2°C too warm. They entirely missed the 1975 cooling trend, which culminated in the coldest NINO3 SST in the entire data record. This failure is present even when the model is forced by observed winds, as in the run creating initial conditions (see, for example, the initial SST for the January 1976 forecast in Fig. 2). On the other hand, the model never predicts an El Niño for 1975, although at the time observers saw enough precursors of an event early in the year to announce an El Niño watch.

It is important to know whether the model's ability to predict whether an El Niño will occur is significantly better than chance. Because the distribution of states in the tropical Pacific is strongly bimodal¹, it is straightforward to distinguish El Niño events from non-events. In terms of NINO3 SST we will say there is an El Niño if there is a rise of at least 2°C within 12 months, leading to an anomaly of at least $+1.0^{\circ}\text{C}$ for at least 3 months. These criteria are derived from the observed events; the set of forecasts would score similarly for any other criteria able to separate observed events from non-events.

According to the above criteria, 19 of the 24 forecasts are correct. (The forecasts would be judged to be less successful if more detailed agreement with observation were demanded.) January 1971, April 1971, October 1975 and April 1981 fail to predict the upcoming event, and April 1978 falsely predicts an El Niño in 1979. Of the five failures, three are from April starts. In the spring the Intertropical Convergence Zone is closest to the Equator and the SST in the east is warmest. As a result the coupling between model atmosphere and ocean is such that noise in the initial state is easily amplified, leading to poor results. The real ocean-atmosphere system is also least predict-

able in the spring: it is the time when the Southern Oscillation index shows the smallest correlation with its future value⁴¹.

Meteorological forecasts are typically evaluated relative to climatology or persistence, but either overestimates the predictive skill for infrequent events like El Niño. Table 1 offers another standard: the random guess. It may seem artificially favourable to compare to a guessing strategy which makes no apparent use of the known 3-4-yr quasi-periodicity of the ENSO cycle²; however, we have forecast only for periods 3 or 4 years apart, when events are seemingly due. Hence the occurrence or non-occurrence of an event in any of the years in our sample is roughly equally likely, so that flipping a coin is a reasonable guessing strategy.

In order to establish a rigorous confidence level, the number of independent forecasts must be known. A precise determination of independence would require many more model runs, but estimates adequate for present purposes can be readily made. We treat forecasts that are 3 months apart as independent, based on the observation that qualitative behaviour (such as incorrect forecasts or maintaining high SST in the year after an event) rarely persists longer. Doing this probably overestimates the confidence level. At the other extreme, a very conservative estimate is obtained by assuming that the forecasts within each set are highly correlated. Then there are only four independent forecasts, each given by the majority of the six within each set. As all four are correct, there is 1 chance in 16 of doing as well by coin flipping. Our most plausible estimate is based on the fact that, whereas indices of the Southern Oscillation can be highly correlated for many months, the months preceding April of a given year correlate poorly with months following it⁴¹. This suggests treating the first October, January and April of a set as one forecast, independent of a second forecast derived from the subsequent July, October and January. There are now eight independent forecasts, the last seven of which are correct. The probability of guessing this successfully is ~ 0.04 .

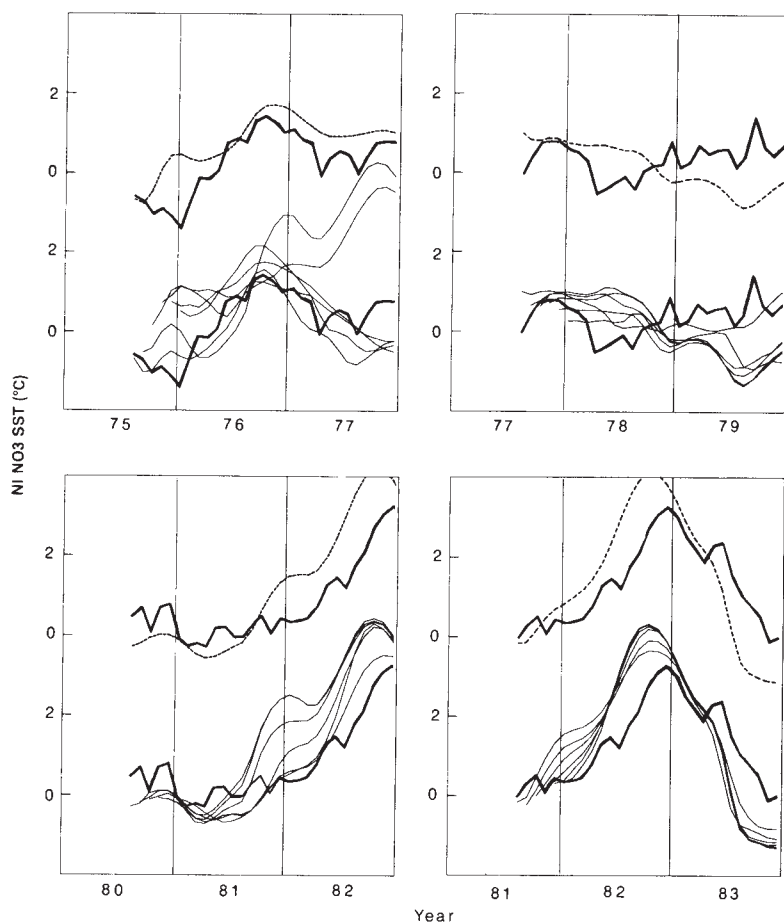


Fig. 3 SST anomalies ($^{\circ}\text{C}$) for selected periods, averaged over the NINO3 region. Light curves, values from forecasts initiated in the six successive months from August to the following January. Dashed curve, average of the six (the consensus forecast); heavy curves, observed values (note repetition of vertical scale).

Another measure of model performance is obtained by considering events and non-events separately. Altogether, 14 of 18 occurrences of El Niño and 5 of 6 non-occurrences are forecast correctly. The joint probability of getting at least that many of each type right by an optimal random strategy is 0.004. (The optimal random strategy assumes *a priori* knowledge of the number of events and non-events in the sample, as well as the score to beat.) The corresponding joint probabilities for the 'conservative' and 'most plausible' cases are 0.06 and 0.04, respectively.

Forecasts started in the same calendar month in different years differ only in their initial conditions. Their success supports the assertion that the future evolution of ENSO is implicit in the initial conditions. Consider, for example, the eight 1-yr forecasts initiated in January. Even if one knew in advance that there were three El Niño events and five non-events, the chance of matching the model performance by guessing all eight correctly is only 1 in 56.

Rather than considering forecasts at 3-month intervals, we now examine a more useful forecasting strategy. A forecast is initiated in each of the 6 successive months from August to January, to obtain the prediction of the ensemble 1 and 2 years ahead. Table 2 summarizes the results of doing this in every year for which we have data (1970–1984), with the exception of the three El Niño years in the sample. A few examples are shown in Fig. 3. There are twelve sets of 1-yr forecasts: in nine of these all 6 months agree so the interpretation of the model prediction is unambiguous. In all nine the prediction is correct. By the rather stringent standard which scores the other three as wrong, the probability of doing as well or better by chance is 0.07.

It would be more accurate to say that the three latter forecasts provide unclear guidance. In two of the cases, where there was no event in the following year, half of the months forecast

correctly and the other half did not. The remaining case is the set from August 1975 to January 1976. As can be seen from Fig. 3, the model actually does a good job of predicting the odd event to come. However, four times out of six our temperature rise criterion is not met because the model SST is too warm at the beginning of 1976. One might quibble with our scoring system, but it would undoubtedly have been difficult to interpret such a forecast at the time.

As might be expected, the 2-yr forecasts are less successful: in five cases all six individual forecasts are correct; in three cases five of six are correct; in the remaining cases one, two or three are correct. Counting eight of the eleven as successes gives

Table 2 Success rates of 6-month sets of forecasts

Forecast from	No. correct (out of 6)	
	1-yr	2-yr
1970	6	5*
1971	6*	1
1973	6	3
1974	6	6*
1975	2*	5
1977	6	6
1978	6	6
1979	6	2
1980	6	6*
1981	6*	6
1983	3	5
1984	3	?

Summary of the sets of forecasts initiated in the six successive months from August to January of each year from 1970 to 1984 (excluding El Niño years). For example, in the first line of the table, the forecasts are from August 1970 to January 1971; the 1-yr forecasts are for 1971 and the 2-yr forecasts are for 1972.

* Year of El Niño event.

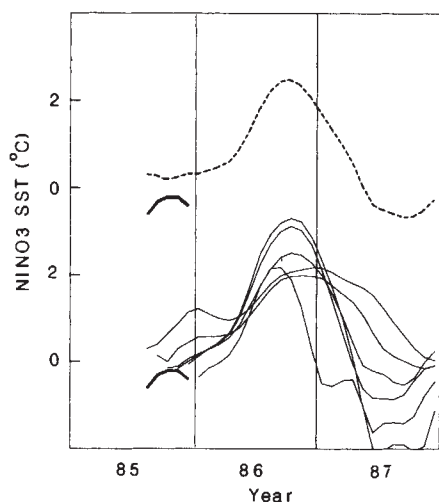


Fig. 4 Forecast for 1986 of SST anomalies ($^{\circ}\text{C}$) in the NINO3 region. Light curves, values from forecasts initiated in the six successive months from August 1985 to January 1986. Dashed curve, average of the six forecasts; heavy curves, observed values (note repetition of vertical scale).

an 89% confidence level. The most consistent failure is the 1971 set, in which all forecasts predicted the 1972 E Niño but five of the six had the warming persist through 1973. Four of the 1979 forecasts predicted an event in 1981, a year early. The 1983 set is the most ambiguous; note that for the years since the outsized 1982 event both the 1- and 2-yr forecasts have been inconsistent.

We present another case, which cannot be evaluated at the time of writing: the prediction for 1986. Figure 4 shows the forecasts initiated in August 1985 to January 1986: all six predict an El Niño event. The amplitude of the consensus forecast suggests it will be a moderate event, not as strong as the events of 1982, 1972 or 1957. However, the only other moderate event in our study was the peculiar 1976 El Niño; experience with the moderate event of 1965 and the even weaker 1969 one might help in interpreting the present case. For the three events in our sample (1972, 1976 and 1982) the consensus overstated the maximum NINO3 SST anomaly amplitude by 30% (more precisely, by 28%, 36% and 28%, respectively). Re-scaling the 1986 forecast accordingly indicates an amplitude of 1.9°C , well below the 3.2°C of 1982 or the 2.5°C of 1972, but above the 1.4°C of 1976.

How much confidence should be placed in the prediction of a 1986 El Niño? As with all forecasting, there exists the possibility that the present case will put pressure on previously untested weak points in the model. Forecasts since the 1982–83 event have been problematical in some respects, possibly reflecting a shift in circulation regime which could adversely affect model performance. Although all of the 1985 forecasts predict an event for 1986, none of the 2-yr forecasts from 1984 predicts it. This is unprecedented among the limited set of cases we have studied. Also, both the 1983 and 1984 1-yr forecasts lack the consistency which is typical of earlier years. Nonetheless, in no previous case in which all six 1-yr forecasts agreed were they incorrect. Moreover, all eleven forecasts from March 1985 to January 1986 give the same result.

Discussion

The ability of the model to predict at long lead times lends support to the theory outlined above⁵, especially the idea that the ENSO cycle owes its existence to interactions in the tropical Pacific. Although the thermal anomalies in the upper layers of the tropical ocean are crucial to the long timescale of the cycle, the ocean alone could not sustain the cycle from event to event. It is an essential part of the ENSO cycle that the two-way coupling between tropical atmosphere and ocean is active in

both the El Niño and non-El Niño phases of the cycle.

The results presented here have significant implications for the future prospects of ENSO prediction systems. It seems clear that the predictability limit for ENSO is years rather than weeks or months. We cannot yet state it more precisely, and it cannot be calculated from forecasts alone because the intrinsic lack of predictability of the coupled ocean-atmosphere system is not the only reason for inaccurate forecasts; poor data and model errors also contribute. We presume these failings account for the cases where the forecasts from many consecutive months are both consistent and wrong (for example, the forecasts for 1973 from 1971). It is also likely that the predictability is highly variable: at some times the future evolution of the coupled system is insensitive to small changes; at others, the system passes so close to a bifurcation point that even small-amplitude disturbances may alter its future behaviour substantially, perhaps making the difference between El Niño and non-El Niño states. We conjecture that the latter times are those for which forecasts from consecutive months are inconsistent (for example, the forecasts from 1983). Here we are implicitly assuming that month-to-month variations in initial conditions are sufficient to reveal all of these uncertain times. Further studies may reveal a better indicator of periods of unpredictability.

In view of the sparsity of the observations used to produce model initial conditions, the success of the forecasts is somewhat surprising, and certainly encouraging. Even without any new breakthroughs in understanding, it should be possible to lengthen the lead time of the model predictions, increase their reliability and improve the forecast of features more subtle than NINO3 SST.

Much more could be done to process the initial data for the purpose of forecasting. All operational meteorological forecast models have very elaborate procedures for data assimilation and model initialization. The only observations used here are ship reports of surface winds. Available observations of SST, currents and ocean thermal structure should be incorporated in the initial fields. Enhancement of the observing system would increase forecast skill: we expect remote sensing of surface winds from satellites to be especially beneficial.

The model used here was developed for theoretical studies, rather than forecasting. It was deliberately simplified in order to isolate the physics believed to be essential to ENSO, putting it at a level of complexity comparable to numerical weather prediction models of 30 years ago. Because our model is based on physical principles, it can be elaborated to increase the fidelity of its simulations. It seems clear that with better observing networks and more refined models, operational climate forecasts of definite social and economic benefit can be expected in the near future.

We dedicate this paper to the memory of Dr Adrian Gill in appreciation of the encouragement he gave this work, and, more broadly, in recognition of his essential contributions to the understanding of ENSO. We thank Professor J. J. O'Brien and David Legler of Florida State University for providing the surface wind analysis, Dr George Philander for his stimulating comments on an earlier draft, and Professor Harold Sackowitz for advice on statistical matters. This work was supported by grants NA-84-AA-D-0031 from the US TOGA Office of NOAA, NA-84-RAD-05082 from EPOCS/NOAA, and NAGW-582 from NASA. This is Lamont-Doherty Geological Observatory contribution 4004.

Note added in proof: No indication of El Niño is apparent as of the end of May 1986. There is no known precedent for an event to begin later than June.

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Secular variation in carbon isotope ratios from Upper Proterozoic successions of Svalbard and East Greenland

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Analyses of stratigraphically continuous suites of samples from Upper Proterozoic sedimentary successions of East Greenland, Spitsbergen and Nordaustlandet (Svalbard) provide an approximation to the secular variation in carbon isotope ratios during a geologically and biologically important period of change from around 900 million years ago to the beginning of the Cambrian period. Late Riphean carbonates and organic material show a stratigraphically useful pattern of enrichment in ^{13}C relative to Phanerozoic or earlier Proterozoic samples. Isotopic compositions of isolated samples from other localities are consistent with a worldwide extended interval of enhanced organic burial and consequent net survival of oxidized material, probably O_2 , just before the initial radiation of metazoans.

SECULAR variations in the carbon isotopic composition of sedimentary carbonates and organic materials have been determined with reasonable temporal precision for most of the Phanerozoic eon, and these data have assumed a significant role in discussion of Phanerozoic environmental history^{1–4}. Analyses of Precambrian samples show that on a gross scale the earlier record of carbon isotopic variation is not dissimilar to that of the past 570 Myr (ref. 5); however, insufficient sampling density and a lack of stratigraphic control have hindered attempts to continue the curve representing of carbon isotopic abundances in marine carbonates backward into the Proterozoic.

We report here the results of carbon isotopic analyses of stratigraphically continuous suites of samples collected from Upper Proterozoic sedimentary successions in Nordaustlandet (Svalbard), northeastern Spitsbergen and central East Greenland. Stratigraphic position, palaeoenvironmental setting and petrography have been determined for each sample, and a degree of biostratigraphic control is provided by acritarch assemblages that occur in all three sequences. The sections, which represent widely separated localities within a single sedimentary basin, show smoothly covariant carbon isotope records for both carbonate and organic carbon. Here we consider three questions bearing on the interpretation and significance of these isotopic variations:

(1) Are they primary (that is, reflective of changes in the isotopic composition of carbon available for biosynthesis of organic matter)?

(2) Are they of global extent (representative of changes in

the isotopic composition of oxidized carbon in the world ocean)?

(3) Do they reflect changes in the Earth's surface environment, especially changes in O_2 concentration, which may have been important in late Proterozoic biological evolution?

Geological setting

The close lithostratigraphic similarities between the Upper Proterozoic and Lower Palaeozoic sequences of the East Greenland Caledonides and eastern Svalbard have long been appreciated⁶. The Spitsbergen, Nordaustlandet and East Greenland sections each contain 7,000–8,000 m of unmetamorphosed, predominantly shallow marine sedimentary rocks, comprising two quartz-arenite-carbonate successions separated by an interval of glaciogenic sedimentation^{7–14}. Table 1 summarizes the nomenclature of the sampled rock units, and stratigraphic columns are shown in Fig. 1.

Thick sequences of cross-bedded and rippled sandstones with interbedded rippled, often carbonaceous shales grade upward into carbonates with minor siliciclastic intercalations. The limestone and dolostone successions contain abundant stromatolites, cross-bedded oolites and pisolites, and intraformational conglomerates, all of which indicate intertidal to shallow subtidal marine deposition. The carbonates are overlain abruptly by a diamictite-bearing siliciclastic sequence, which is in turn overlain by a quartz-arenite-carbonate sequence of Cambro-Ordovician age. Microfossil assemblages^{15–17} and stromatolites¹⁸ indicate that the earlier siliciclastic and carbonate sequence was deposited ~900–700 Myr ago, during the late Riphean interval,

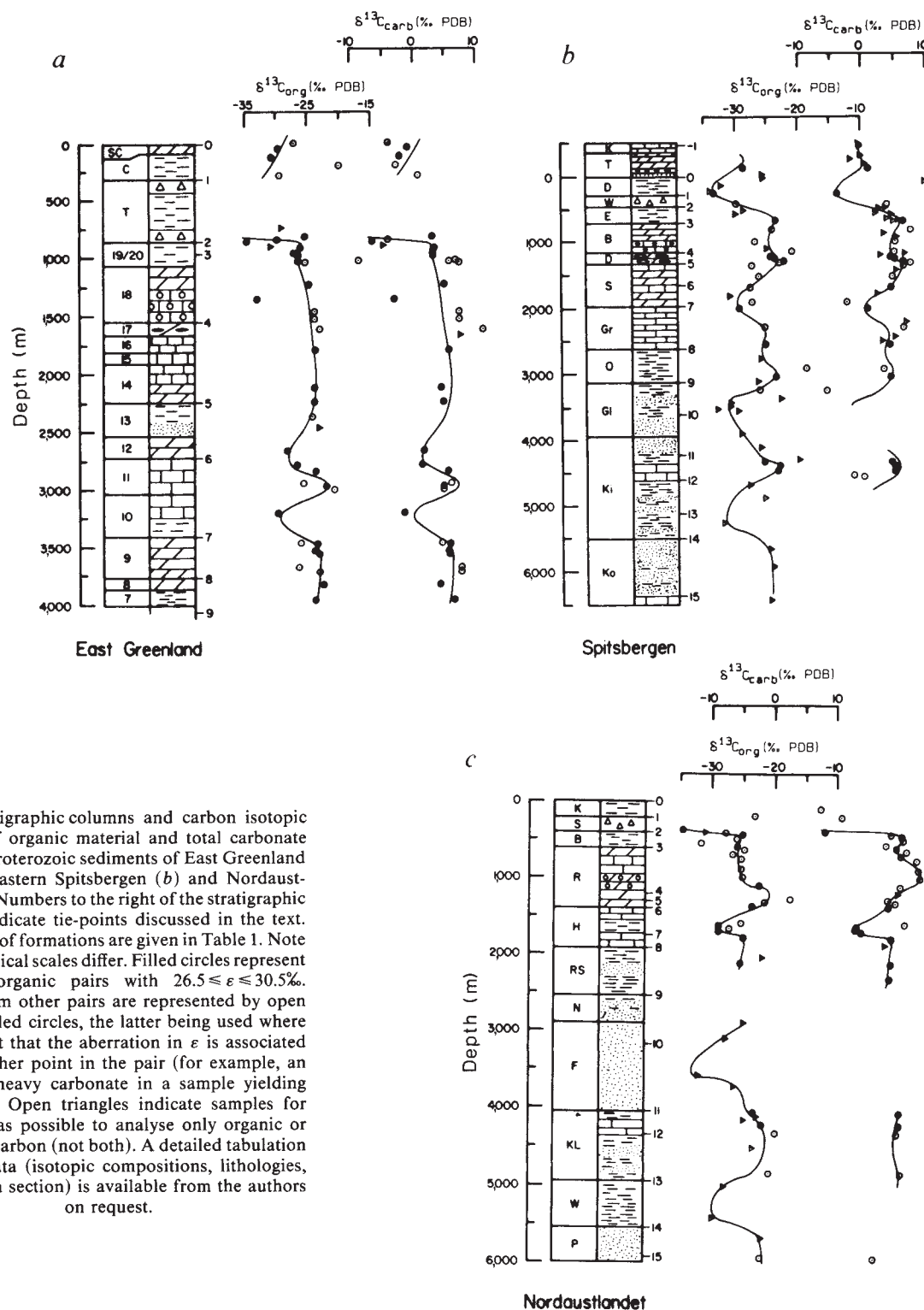


Fig. 1 Stratigraphic columns and carbon isotopic analyses of organic material and total carbonate from late Proterozoic sediments of East Greenland (a), northeastern Spitsbergen (b) and Nordaustlandet (c). Numbers to the right of the stratigraphic columns indicate tie-points discussed in the text. Full names of formations are given in Table 1. Note that the vertical scales differ. Filled circles represent carbonate-organic pairs with $26.5 \leq \epsilon \leq 30.5\%$. Results from other pairs are represented by open and half-filled circles, the latter being used where it is evident that the aberration in ϵ is associated with the other point in the pair (for example, an unusually heavy carbonate in a sample yielding $\epsilon \geq 30.5\%$). Open triangles indicate samples for which it was possible to analyse only organic or carbonate carbon (not both). A detailed tabulation of these data (isotopic compositions, lithologies, locations in section) is available from the authors on request.

and that the diamictite-bearing sequence is Vendian (670–570 Myr) in age. Hiatuses corresponding in time to the latest Riphean and late Vendian to earliest Cambrian occur at the base and top, respectively, of the diamictite-bearing succession¹⁴. (In Greenland, the stratigraphic break appears to occur not at the Eleonore Bay Group/Tillite Group boundary, but within Bed 19 of the Eleonore Bay Group.)

Petrographic studies reveal further strong parallels in the histories of the Greenland and Svalbard sequences¹⁹, and palaeontological analyses show comparable patterns of microfossil distribution^{15–17,20,21}. Based on these observations, 16 tie-points have been identified which can be correlated lithos-

trigraphically with high confidence among sections: these are shown in Fig. 1 (the tie-point at the Precambrian/Cambrian boundary is designated zero, and numbers increase down-section). The close correlations show that the three successions were deposited contiguously along the margin of a single marine basin²². The distance separating the most distant of the successions at the time of deposition was at least 650 km, and possibly much greater. The East Greenland-Svalbard basin apparently constituted the western margin of the expanding Iapetus Ocean during the Cambrian, but debate continues as to whether the thick underlying Upper Proterozoic sequence accumulated intracratonically or on an early-formed passive margin^{22,23}.

Chemical and isotopic analyses

Field specimens were cleaned, fragmented, etched and ground²⁴. Carbon dioxide was produced from carbonates by treatment with H_3PO_4 (density 1.89 g ml^{-1}) at 50°C for 48 h (ref. 25). For determination of the abundance and isotopic composition of organic carbon, sample powders were treated with hot, concentrated HCl in order to remove carbonates, then mixed with CuO and sealed in evacuated quartz tubes for combustion at 850°C for 2 h. Recovery and purification of CO_2 formed by combustion of organic matter allowed both quantitative and isotopic analysis. This procedure was tested by application to 49 samples previously analysed by accepted techniques²⁴, and yielded accurate results for samples similar to those analysed in this work. Isotopic compositions of CO_2 were determined by conventional mass spectrometric techniques²⁶ and are expressed relative to the PDB standard: $\delta = [(R_x/R_{\text{PDB}}) - 1] \times 10^3$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ and the subscript x designates the analyte. For compactness, we employ δ_c and δ_o to refer to the carbon isotopic compositions of carbonate and organic carbon, respectively.

Results and discussion

Isotopic variations. Figure 1 summarizes the results of the isotopic analyses: it is evident that the abundance of ${}^{13}\text{C}$ varies in both carbonate minerals and organic material. Such variations can be primary (reflecting changes in the isotopic composition of carbonate in the water column beneath which the sediments initially accumulated) or secondary (reflecting bacterial reworking of organic material, precipitation and admixture of new carbonates, or isotopic exchange with species in solution). Consideration of the significance of these isotopic variations requires resolution of primary and secondary effects.

Recognition of truly primary isotopic compositions and perfect elucidation of all secondary variations is difficult in the best of circumstances²⁷. However, no secondary processes are known (or are, for that matter, conceivable) which always shift the isotopic compositions of carbonate and organic carbon in the same direction at the same rate. Instead, secondary processes commonly alter the isotopic composition either of organic carbon²⁸ or of carbonate carbon²⁹, and the magnitudes of the isotopic shifts are controlled by different and unrelated factors. Primary processes (biosynthesis of organic material and formation of carbonate in equilibrium with dissolved CO_2), on the other hand, tend to maintain a constant isotopic difference between carbonate and organic carbon. Systematic consideration of the isotopic fractionation ($\epsilon \approx \delta_c - \delta_o$) can therefore assist

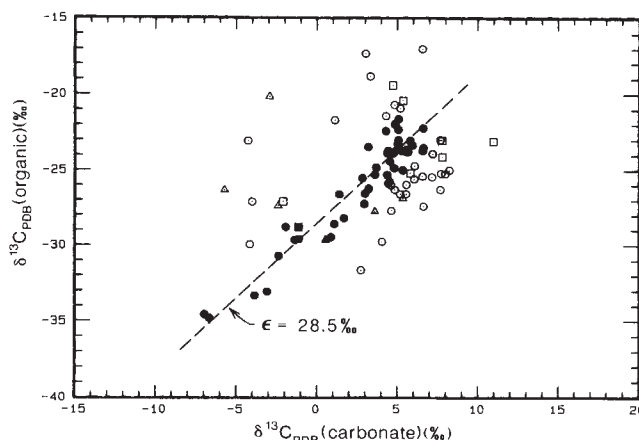


Fig. 2 Carbon isotopic compositions of samples in which it was possible to analyse both organic and inorganic phases. Squares, samples in which the concentration of organic carbon was $<0.2 \text{ mg C per g sample}$; triangles, samples containing $<10\%$ carbonate (as calcite); filled symbols, samples with $26.5 \leq \epsilon \leq 30.5\%$.

in the resolution of primary and secondary isotopic variations.

The data include 92 pairs of measurements from which ϵ values can be computed; the carbon isotopic compositions of carbonate and organic materials in these samples are compared in Fig. 2. The mode of the distribution of ϵ values lies near 28.5% (dashed line in Fig. 2). Many of the carbonate-organic pairs yielding $\epsilon > 30.5\%$ or $\epsilon < 26.5\%$ contain $<0.2 \text{ mg organic C per g sample}$ or $<10\%$ carbonate; because of their low carbon concentrations, the isotopic compositions of these samples are particularly vulnerable to diagenetic alteration. We conclude that samples yielding $\epsilon = 28.5 \pm 2.0\%$ preserve primary carbon isotopic compositions within a few per mil. We do not contend that isotopic shifts due to diagenesis are absent in these sections, and will return to this point below. We do suggest, however, that measurement of both organic and inorganic carbon isotopic compositions greatly reduces the chance of misinterpretation and allows the recognition of major features in the isotopic records compiled in this work.

Correlations between sections. Comparison of variations observed in the different sections suggests that all three record the same set of primary variations. For example, all are depleted in ${}^{13}\text{C}$ in the Vendian and show a general pattern of ${}^{13}\text{C}$ enrichment during the late Riphean. The lithostratigraphic tie-points

Table 1 Stratigraphy and correlation among sections

East Greenland ^{13,14}		Northeastern Spitsbergen ⁷⁻¹¹		Nordaustlandet ¹²		Tie-points* and chronostratigraphy	
Group	Formation	Group	Formation	Group	Formation		
Tillite	{ Spiral Creek Canyon Tillite	Oslobreen	{ Kirtonryggen Tokammane	Gotia	{ Klackberget Sveanor Backaberget	-1 540 Myr Cambrian	
		Polarisbreen	{ Dracoisen Wilsonbreen Elbobreen			0 560 Myr (hiatus) 590 Myr Vendian	
		Akademikerbreen	{ Backlundtoppen Draken Svanbergfjellet Grusdievbreen			Roaldtoppen	Ryssö Hunnberg
Eleonore Bay	{ Bed 19/20 : Bed 8 Bed 7		Veteranen	{ Oxfordbreen Glasgowbreen Kingbreen Kortbreen	Celsiusberget	Raudstup/Sälodd Norvik Flora	8 800 Myr Upper Riphean
						Franklinsundet	Kapp Lord Westmanbukta Persberget

* Summarizes placement of rock units within chronostratigraphic units and indicates lithostratigraphic tie-points for which ages have been estimated by the authors. Precise placement of tie-points within each section is shown in Fig. 1.

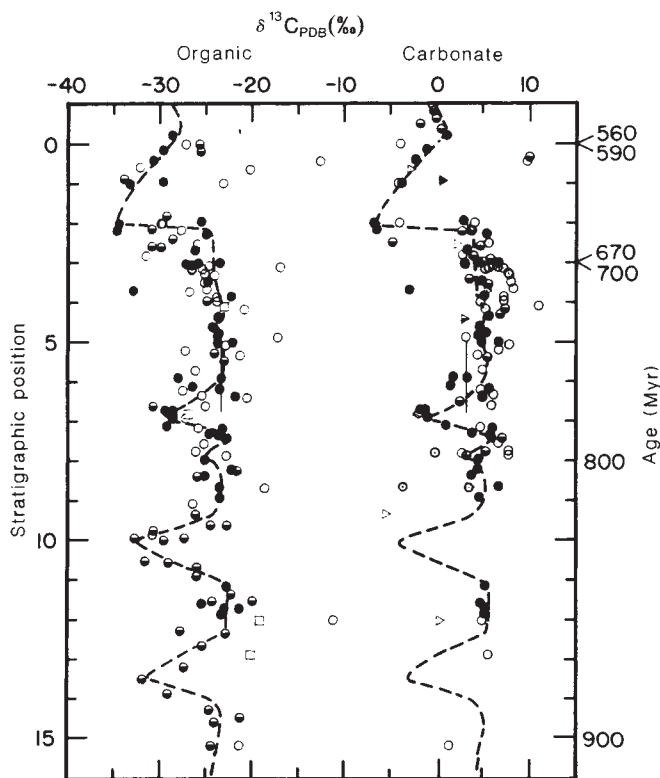


Fig. 3 Isotopic analyses from Fig. 1 pooled and re-plotted on correlated depth scale (numbers at left refer to the tie-points shown in Fig. 1; the scale is thus not linear with respect to either time or thickness of section). Symbols as in Fig. 2 except that half-filled symbols designate samples for which analysis of only one carbon phase (carbonate or organic) was possible. Vertical lines near depth = 6.0 represent a single sample for which stratigraphic placement was uncertain.

already described can be used to plot data from all sections on a single graph, facilitating investigation of isotopic correlations.

In Fig. 3, the ordinate ('stratigraphic position') represents the sequence of tie-points and is thus a scale related to depth. Isotopic compositions of samples collected at stratigraphic levels equivalent to tie-points have been plotted at integer values; others have been placed along the ordinate by linear interpolation between tie-points within each section. The dashed lines are separated by 28.5‰, and were derived by overlaying the carbonate and organic carbon isotopic records (offset by 28.5‰) and considering the trend of both sets of data. The close parallel between the organic and inorganic records is clearly visible in the upper part of the section. Carbonates are rare in the lower part of the section, but strong evidence for additional isotopic variations appears in the organic carbon record. We emphasize that our stratigraphic correlation of the three sections was completed independently of the isotopic analyses; thus, the high degree of congruence seen in Fig. 3 demonstrates the potential of chemostratigraphy based on carbon isotopic compositions for accurate intra-basinal correlation of late Proterozoic sedimentary sequences.

The record of isotopic abundances summarized by Fig. 3 encompasses >300 Myr: the lowermost Cambrian, the Vendian and the Riphean to ~900 Myr. Compared with isotopic compositions prevailing during most of the Phanerozoic (and earlier in the Proterozoic, as far as can be determined³⁰), its principal features are a general pattern of strong ^{13}C enrichment during the late Riphean, an extended interval of ^{13}C depletion in the Vendian and, apparently, several episodes of near-'normal' isotopic compositions in the late Riphean.

As enrichment of ^{13}C in carbonates is so prominent in the isotopic record summarized in Fig. 3, the process of

methanogenic diagenesis should be considered. Fermentative processes yielding methane, often associated with the formation of carbonates enriched in ^{13}C (ref. 29), apparently have no effect on the isotopic composition of coexisting organic matter. Thus, methanogenic diagenesis shifts points to the right on a graph like that shown in Fig. 2, and it is conceivable that carbonates represented by some of the open points to the right of the broken line in Fig. 2 have been diagenetically enriched in ^{13}C . It is, however, extremely unlikely that this process is responsible for the general enrichment of ^{13}C observed in these sections. To sustain that interpretation, it would be necessary to suggest that, in spite of wide variations in facies (tidal-flat microbial mats, cross-bedded oolites, subtidal dolomitic shales) and carbonate abundance, methanogenesis had, over more than 100 Myr, uniformly affected all of the carbonates that we associate with primary variations in isotopic abundance (filled circles in Fig. 2).

Isotopic compositions within the Vendian Gotia, Polarisbreen and Tillite groups are more variable, reflecting the re-deposition of late Riphean carbonates as detritus³¹, as well as primary variations, but horizons of notably 'light' carbon can be correlated among sections. Cambrian values, obtained only from the Spitsbergen section, range from -1.8 to +1.1‰, similar to previously published values for Lower Palaeozoic rocks¹. In Spitsbergen, the base of the Cambrian is recognized by the first appearance of Lontova-type acritarchs and diverse trace fossils of Cambrian aspect; our Lower Cambrian carbonates are associated with *Salterella* and late-*Holmia*-age acritarchs²¹.

Mechanism and scale of primary variations. What might account for the enrichment of ^{13}C in the Upper Riphean sediments of Svalbard and East Greenland? On a basin-wide scale, enrichment of heavy isotopes can be caused by widespread and persistent evaporitic conditions. Isotopically heavy carbonates ($\delta_c \approx +3.0$ – 5.6 ‰) are forming today by this mechanism in Shark Bay, Western Australia (J. Ferguson, L. A. Plumb and M. R. Walter, unpublished data), and the heavy carbon in the sections studied here might have a similar origin (although evaporation does not always lead to marked ^{13}C enrichment; see ref. 32). Two arguments militate against this, however. First, evaporites and indirect sedimentological evidence of evaporitic environments are conspicuously absent in these sections. Second, a clearly evaporitic sequence from the Proterozoic of northwestern Greenland (the Narssarssuk Formation³³) has yielded $0.65 \leq \delta_c \leq 2.65$ ‰ (mean of 12 samples = 1.65‰), somewhat heavy relative to early Palaeozoic mean values, but not comparable to the values recorded for Svalbard and East Greenland sediments.

Primary shifts in the isotopic composition of sedimentary carbon can also be due to changes in the isotopic composition of water-column carbonate caused by preferential biological fixation of ^{12}C into organic material^{28,34}. Enhanced rates of burial of organic material lead to greater-than-normal enrichment of ^{13}C in carbonates³⁵.

The scale of these effects can be global or basinal, and assessment of the geochemical significance of isotopic shifts depends strongly on resolution of these alternatives. In a restricted basin, fixation and burial of organic carbon will cause enrichment of ^{13}C in carbonates if diffusion from the atmosphere and patterns of circulation are unable to maintain isotopic equilibrium with the open ocean. An effect of this kind has been suggested³⁶ as the explanation for the high levels of ^{13}C enrichment in the early Proterozoic Lomagundi Group, Zimbabwe. Heavy carbonates might alternatively be due to enrichment of ^{13}C in carbonate, not in a restricted basin, but throughout the world ocean. An enhancement in the global rate of burial of organic carbon is required to bring about this effect. In the case of the Lomagundi Formation, correlation with coeval sequences from other areas is difficult, so it has not proved possible to resolve the issue by determining whether enrichment was global. Relatively heavy carbon in the Permian, on the other hand, is demonstrably widespread^{1,32}.

The possibility that a similar global enrichment occurred in

Table 2 Summary of previous analyses of late Proterozoic carbonates

Rock unit*	Age (Myr)†			δ_c	s.d.	n	Ref.
	Min.	Prob.	Max.				
Yakutsk, Siberia, Yudoma Fm	570	575	670	2.2	1.1	6	38
Yakutsk, Siberia, Yudoma Fm	570	585	670	-0.6	1.0	17	38
Nama Group, Schwarstrand Subgroup	570	590	650	-3.1	0.8	7	57
Yakutsk, Siberia, Yudoma Fm	570	595	670	-3.9	0.7	6	38
Nama Group, Kuibis Subgroup I	570	610	650	-0.9	1.3	5	57
Nama Group, Kuibis Subgroup II	570	610	650	2.2	1.2	8	57
Morocco, Serie Lie de Vin	570	610	670	-2.5	1.3	14	39
Adelaide Geosyncline, Marinoan I	570	650	700	7.4	3.6	3	30
Adelaide Geosyncline, Marinoan II	570	650	700	-6.3	2.4	5	30
Adelaide Geosyncline, Nuccaleena Fm	570	650	700	-2.5	0.7	2	58
Kimberley Reg., Late Proterozoic I	570	650	700	-2.9	0.3	5	58
Kimberley Reg., Late Proterozoic II	570	650	700	0.9	1.3	5	58
Amadeus Basin, Pertatataka Fm	625	675	700	5.2	0.5	2	30
Flinders Range, Umberatana Gp	650	675	725	3.3	2.5	3	57
Eleonore Bay Fm, Bed 18	650	700	725	5.2		1	57
S. Norway, Hedmark Gp, Biri Fm	668	700	750	1.7	0.4	11	59
Eleonore Bay Fm, Beds 14-16	700	720	740	5.7	1.0	4	57
Morocco, Dolomite Inferieur	670	735	800	(-2.1	to 6.9)	10	39
Eleonore Bay Fm, Beds 12-13	720	740	760	4.5	0.7	3	57
Adelaide Geosyncline, Sturtian	650	750	750	2.4	0.8	6	30
Adelaide Geosyncline, Tapley Hill Fm	650	750	750	1.8		1	58
Adelaide Geosyncline, Tapley Hill Fm	650	750	750	-3.0	0.6	10	60
Adelaide Geosyncline, Tapley Hill Fm	650	750	750	-0.7	0.4	2	61
Adelaide Geosyncline, Tapley Hill Fm	650	750	750	-0.2	2.3	20	62
Kimberley Region, Landrigan Tillite	650	750	750	-5.5		1	58
Katanga, Lower Kundelungu Series	640	750	840	1.3		1	57
India, Chattisgarh Basin	700	750	850	3.9	1.1	5	57
Eleonore Bay Fm, Bed 9	750	775	800	5.3	0.2	2	57
Taoudenii Syncline, Atar Gp I-11	725	775	825	-0.4		1	57
Kango and Malmesbury Group I	600	775	950	6.6	2.7	4	57
Kango and Malmesbury Group II	600	775	950	-0.3	1.1	11	57
Otavi Gp, Tsumeb Subgroup I	750	780	800	5.1	2.8	6	57
Otavi Gp, Tsumeb Subgroup II	750	780	800	-1.0	0.2	3	57
Taoudenii Syncline, Tifouka Gp, I12	650	800	950	0.7		1	57
Otavi Gp, Upper Abenab Subgroup	750	810	830	2.5	0.0	2	57
Amadeus Basin, Bitter Springs Fm	750	825	900	3.4		1	5
Amadeus Basin, Bitter Springs Fm I	750	825	900	3.5	1.6	8	30
Amadeus Basin, Bitter Springs Fm II	750	825	900	-0.9	2.1	6	30
Taoudenii Syncline, Atar Gp, I-9	725	825	925	0.5	1.9	2	57
Burra Gp, Skillogalee Dolomite	750	850	850	-2.0		1	57
Burra Gp, Skillogalee Dolomite	750	850	850	4.6		1	5
Burra Gp, Skillogalee Dolomite	750	850	850	2.3	1.0	2	‡
Death Valley, Beck Springs Dolomite	800	850	1200	4.2	0.3	13	65
Taoudenii Syncline, Atar Gp, I-7	800	870	900	0.1		1	57
Adelaide Geosyncline, Burra Gp I	840	875	930	-4.5		1	30
Adelaide Geosyncline, Burra Gp II	840	875	930	4.2	0.7	4	30
Katanga, Mwashia Group	840	875	930	-3.5		1	57
Taoudenii Syncline, Atar Gp, I-6	850	880	920	-2.2		1	57
Taoudenii Syncline, Atar Gp, I-5	850	890	925	1.0	0.4	2	57
Katanga, Lower Roan Gp I	930	1,000	1,200	5.7	1.6	2	57
Katanga, Lower Roan Gp II	930	1,000	1,200	-1.1	2.7	11	57
Lake Superior Basin, Sibley Group	1,000	1,050	1,300	-0.3		1	57
Adelaide Geosyncline, Eurelia Beds	1,000	1,060	1,100	3.5	0.8	30	64
Adelaide Geosyncline, Willouran	1,000	1,060	1,100	2.7	1.2	4	30
Glacier National Park, Middle Belt	910	1,075	1,400	0.5		1	65
Oakway Group	850	1,150	1,450	-1.1		1	57
Arizona, Apache Gp, Mescal Limestone	1,150	1,200	1,500	3.1	0.4	16	66
India, Cuddapah Basin	1,100	1,400	1,600	-0.9		1	57
McArthur Basin, HYC (least altered)	1,400	1,640	1,688	-0.3	0.4	7	67
McArthur Basin, McArthur Group	1,590	1,650	1,690	-1.2	1.9	6	30
McArthur Basin, McArthur Group	1,590	1,650	1,690	-1.0	0.9	6	5
McArthur Basin, Wollogorang Fm	1,590	1,650	1,690	-1.2	1.1	7	§
Birrindudu Basin, Bungle Bungle Dol	1,560	1,650	1,750	-0.7	0.3	7	5

* Roman numerals appended to rock units designate separate modes of bimodal distributions.

† Minimum, probable and maximum ages estimated by the authors.

‡ I. B. Lambert, unpublished data.

§ M. J. Jackson and T. H. Donnelly, unpublished data.

the late Proterozoic can be examined by consideration of all previous reports of carbon-isotopic compositions of middle and late Proterozoic sedimentary carbonates from North America, Africa, India and Australia. Figure 4 shows these data and a summary of carbonate isotopic compositions during the Phanerozoic, together with a curve representative of the isotopic compositions observed in this work. The Phanerozoic and late Proterozoic records differ greatly in temporal resolution, numbers of samples and levels of selectivity. We do not suggest that they can be compared directly in all respects, but contrasts are of such interest that some preliminary consideration is in order.

Comparison (Fig. 4) of the curve representative of isotopic compositions observed in this work with points representing all entries in Table 2 shows that these records are consistent, in that both suggest persistent and significant enrichment of ^{13}C in late Riphean sedimentary carbonates, as well as episodes of ^{13}C depletion or normalcy. The scatter of points representative of other rock units around the East Greenland-Svalbard curve is not significant because the stratigraphic positions of many samples are imprecisely defined, and radiometric and biostratigraphic age determinations for many sequences require further work (age uncertainties are estimated in Table 2). Existing data are thus consistent with the idea that the detailed isotopic records observed in this work reflect global trends, but stratigraphic data are insufficient to establish this point in great detail.

Implications. It should be evident that isotopic studies can assist in the reconstruction of stratigraphic relationships. Margaritz³⁷ has shown that systematic variations of the carbon isotopic compositions of sedimentary carbonates can be useful in the correlation of Cretaceous sequences, and Margaritz *et al.*³² have argued that anomalously heavy carbon can be recognized in Upper Permian sections worldwide. Our study of the Svalbard and East Greenland sections indicates that isotopic chemostratigraphy can facilitate correlations within a basin, and comparison of our data with previously published values suggests that stratigraphic patterns of isotope variations will prove useful in making at least gross correlations between sedimentary basins. Particularly encouraging are comparisons between our data and recently published analyses by Margaritz *et al.*³⁸ and Tucker³⁸ of sections from Siberia and Morocco, respectively. All three sections show isotopically light intervals in the Vendian, and the Moroccan data, although poorly constrained stratigraphically, show that apparently Upper Riphean beds near the base of the section have highly variable isotopic compositions similar to those assigned to the time interval 645–775 Myr in this work. (Because of this high variability, a point representative of this portion of the Moroccan section is not shown in Fig. 4.) The Moroccan and Siberian rocks also show a short interval of ^{13}C -enriched carbonate deposition in latest Vendian to earliest Cambrian time, but independent biostratigraphic evidence suggests that rocks of equivalent age are absent in Svalbard and East Greenland²¹. Although inter-basinal resolution may be coarse, any additional means of ascertaining stratigraphic position will be useful for the correlation of Upper Proterozoic sections. The present results also demonstrate that carbon isotopic analyses of organic material can assist in the evaluation of the isotopic record, both in the identification of diagenetic alterations and reconstruction of the record itself.

In addition to their use in stratigraphic correlation, the isotopic data have important implications for phenomena as diverse as palaeoclimatology, end-Proterozoic and early Palaeozoic phosphorite deposition⁴⁰, atmospheric history and biological evolution. Because a global enrichment of ^{13}C in sedimentary carbonates signals an increase in the rate of burial of organic carbon, it also signals a globally significant shift in oxidation-reduction (redox) processes. Reducing potential flowing to the carbon cycle (in order to allow increased burial of organic carbon, the reduced product in the carbon cycle) must have been diverted from another elemental cycle. The geochemically plausible alternatives are the cycles of sulphur (resulting

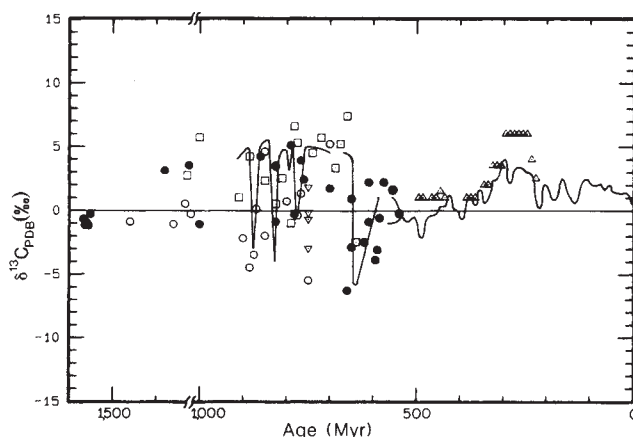


Fig. 4 Carbon isotopic abundances in carbonates with ages of <1,750 Myr. Note compression of age axis, 1,750–1,000 Myr. The continuous curve in the interval 565–0 Myr is the Phanerozoic carbonate curve compiled by Lindh⁶⁸. Triangles in the same interval represent recent measurements of unaltered portions of brachiopods⁶⁹. The broken curve in the interval 915–540 Myr retraces the carbonate line shown in Fig. 3, with allowance for depositional hiatuses at ~700–670 and ~590–560 Myr. Remaining points represent data summarized in Table 2. Open circles, rock units for which a single isotopic analysis is available; open squares, two to four analyses; filled circles, five or more. Triangles at 750 Myr represent the Tapley Hill Formation, which is possibly non-marine⁶⁵.

in an increase in sulphate at the expense of sulphide), iron (resulting in increased precipitation of ferric iron) and oxygen (resulting in accumulation of O_2).

The magnitude of the redox shift can be estimated from the size and duration of the carbon isotopic shift. The flux of carbon in the exogenic cycle in the late Proterozoic is not known. As a point of reference, the present value is $\sim 2.5 \times 10^{19}$ mol C Myr⁻¹ (refs 41, 42). In a geochemical carbon cycle characterized by $\epsilon = 29\%$ (approximately the value observed in this work), precipitation of carbonates with $\delta_c = 0\%$ would correspond (at the modern flux level) to burial of 4×10^{18} mol organic C Myr⁻¹ (=17% of total flux, assuming $\delta = 5\%$ for average crustal C). If carbonates instead have $\delta_c = +5\%$, the rate of burial of organic carbon is doubled, and oxidized products (sulphate, O_2 , Fe^{3+}) must accumulate at a rate equivalent to 4×10^{18} mol O_2 Myr⁻¹. The present atmospheric inventory of O_2 is 3.8×10^{19} mol. The duration of the late Proterozoic interval of precipitation of carbonates with $\delta_c \approx +5\%$ was apparently at least 100 Myr. If the flux of carbon in the exogenic cycle was near modern levels, oxidized material produced during that interval would have been ~10 times the present inventory of O_2 .

The fate of this oxidizing potential is only partly clear. Interpretation of patterns of covariance in the isotopic records of carbon and sulphur suggests that less extreme episodes of oxidation during the Phanerozoic have been largely accommodated by shifts in the redox balance of sulphur⁴. The sulphur isotopic record for the late Proterozoic is far from complete⁴³. Until more is known, quantitative estimates of possible shifts in the redox balance of sulphur will be impossible. We note, however, that the flux of sulphur in the exogenic cycle is about 17 times smaller (on a molar basis^{31,42}) than that of carbon. Even if all of the sulphur returned to the system by weathering and erosion were initially in the form of pyrite, this flux would be equivalent (at modern levels) to 3×10^{18} mol O_2 Myr⁻¹. In the absence of any other sink for oxidizing potential, O_2 would thus be expected to accumulate at a rate of at least 10^{18} mol Myr⁻¹. The flux of carbon in the exogenic cycle may have been decreased during this interval by 'tectonic tuning'⁴⁴, thus slowing the rate of accumulation of O_2 , and a significant portion of the excess might have gone into oxidation^{45,46} of materials newly exposed at

spreading centres⁴⁷⁻⁴⁹, but it seems very likely that the period from 700 to 800 Myr ago was a time of important atmospheric oxygen buildup.

It is interesting to speculate as to what factors controlled the timing of this episode of enhanced burial of organic carbon. Probable eukaryotic phytoplankton appear in the record at least 1,400 Myr ago⁵⁰, and must have been well established as important primary producers long before the interval studied here. Morphological changes observed in microfossils from 850–700 Myr ago (ref. 50) might, however, reflect physiological developments which expanded the range of habitable environments by allowing better control of buoyancy or improved interception of nutrients and light. Furthermore, enhanced rates of burial of organic material might be related to tectonic factors. Many of the best known 'Palaeozoic' geosynclinal sedimentary sequences began to accumulate during this interval, including the Iapetus, Adalaidian and Cordilleran, as well as others in India, China and the Soviet Union⁴⁸. Depositional conditions associated with these developments may well have favoured increased rates of burial of organic carbon⁵¹. At the same time, conditions of high fertility may have been maintained in the marine environment by more efficient recycling of phosphorus within and just below the photic zone. In the modern global ecosystem, biomineralization and the rapid sinking of faecal pellets both remove phosphate from the surface layers of the ocean and tend to enhance its burial; however, faecal pellets and biosynthetic phosphates appear only later in the geological record. If late Proterozoic organic materials were exclusively microbial and sank more slowly, and if grazing heterotrophs did not package phosphate for rapid sedimentation, more efficient use of phosphate would seem probable.

The end of this era of enhanced burial of organic carbon coincides with the major Eocambrian glaciation. The periodic returns to 'normal' carbon isotopic compositions within the era may coincide with earlier glaciations⁴⁸. The occurrence of major glaciations implies the existence of a strong climatic gradient which, in turn, is very likely to have affected circulation within

the ocean, possibly encouraging the development of deep circulation and reducing rates of burial of organic material. By the close of the Varangian glaciation, evolutionary developments had occurred which countered the factors described above, and very high rates of burial of organic carbon were not re-established. It is also possible, of course, that O₂ pressures equal to or higher than present levels were attained and acted to prevent burial of organic material by establishing a particularly aggressive environment.

An increase in atmospheric O₂ levels 700–800 Myr ago is of great palaeobiological interest because many authors have argued that the time of appearance of macroscopic metazoans was constrained by oxygen tensions sufficient to permit epithelial diffusion of oxygen across multiple cell layers, support metazoan exercise metabolism, and permit collagen synthesis (see, for example, refs 52–56). Until now, there have been no geochemical data to support this idea; however, as the conspicuous fossil record of animals begins just after the interval of heavy carbon deposition, it may be that the late Proterozoic isotopic record is supplying the evidence needed to support this concept. Additional data are required to evaluate this possibility. Detailed secular curves for organic and carbonate carbon need to be established for other Upper Proterozoic sections, and these must be complemented by similar curves for sulphur. It is clear, however, that the carbon isotopic record will be an important part of the evidence on which reconstruction of late Proterozoic evolution, environmental history, and their interaction through the carbon cycle will eventually be based.

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Enhancement of high-energy cosmic-ray spectrum by type-II supernovae

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The cosmic-ray spectrum has an intensity enhancement in the energy range 10^{14} – 10^{16} eV per nucleus¹ (Fig. 1). Recent observations of heavy cosmic rays in this energy range indicate that the Ca/Fe ratio may be as large as 10 times the solar value. We suggest that pulsars in type-II supernova remnants are the origin of this component of the cosmic-ray spectrum.

The high-energy enhancement in the cosmic-ray spectrum has been suspected to result from rays originating from pulsars^{2–4}. For example, Karakura *et al.*² explained the feature in the extensive air shower (EAS) energy spectrum in terms of proton-dominated pulsar contributions using the electromagnetic acceleration mechanism proposed by Ostriker and Gunn^{5,6}. This analysis agreed reasonably well with the data available at that time. More recent observations¹ indicate a lower intensity of the enhancement, (one-fifth) of that determined by the old EAS data, and the chemical composition in the flux enhancement range has been controversial with some indirect analyses indicating proton dominance^{7–9} while the others suggest dominance of iron^{10–12}. All of these ambiguities make it difficult to resolve the origin of the enhancement. Recently, however, two new sets of direct observations of chemical composition have led us to propose its origin in type-II supernovae which predicts an overabundance of medium heavy (such as ^{16}O – ^{40}Ca) and/or neutron-rich heavy nuclei (such as, ^{48}Ca).

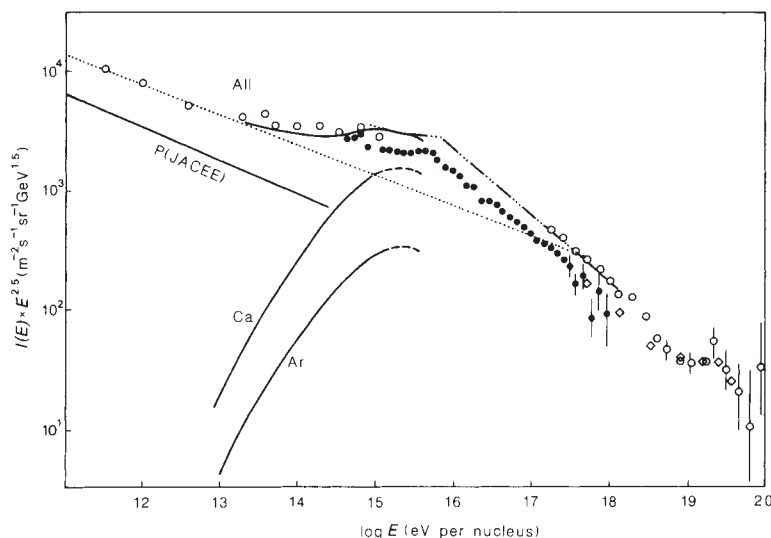
The first of these new observational results were reported by the JACEE collaboration utilizing balloon-borne emulsion chambers^{13–15}. These experiments also showed high-energy enhancement and were consistent with the data of Grigorov *et al.*¹⁶ from the PROTON satellite. Of special interest, the JACEE data showed no anomalous proton, helium, and iron abundances. However, they did observe an overabundance of calcium at high energies (Ca/Fe ≥ 2 above 10^{14} eV per nucleus). They also observed some enhancements of medium-heavy nuclei (from C to Si)^{13–15}. The second set of observations were obtained by the C3 experiment on the HEAO 3 satellite. Data from this experiment allow an analysis of the abundances of the heavy elements relative to iron at energies up to 10^{12} eV AMU^{-1} (ref. 17), and show a dramatic rise in both the calcium and argon abundances at energies above 500 GeV AMU^{-1} (Fig. 2). Although both these results are subject to large statistical and complicated instrumental uncertainties, we find it significant that a calcium overabundance is implied by two independent experiments and suggest that this anomaly has a direct link with type-II supernova as discussed below.

Cosmic rays are generally thought to originate from supernovae, of which there are two types¹⁸; type-I (SN-I), which show no evidence for hydrogen at near maximum light and are observed in all types of galaxies, and the type-II (SN-II), which show hydrogen-rich spectrum with various light curves, and are found only in spiral galaxies.

SN-I are thought to involve a mass-accreting white dwarf where carbon deflagration eventually leads to a total disruption of the white dwarf and a significant amount of ^{56}Ni is produced^{19,20}. The resulting β -decay of the ^{56}Ni to ^{56}Fe provides a satisfactory explanation for changes in the SN-I light curve. The predicted Ca/Fe ratio in the synthesized elements is less than a half of the solar value²⁰. Thus, we can rule out SN-I as a source of the calcium anomaly.

There are, on the other hand, two proposed mechanisms for SN-II: one model, involving 8–11 solar mass stars, invokes core collapse triggered by electron capture on ^{20}Ne and ^{24}Mg in an oxygen–neon–magnesium core²¹. The shock wave, generated when the nuclear matter density core bounces, ejects the overlying mass and leaves a neutron star²². Miyaji *et al.*²¹ found that ^{48}Ca could be a most abundant nucleus when nuclear statistical equilibrium (NSE) is realized above 10^{10} g cm^{-3} . Because the NSE region in their model extends further than the sonic point when the bouncing shock wave is formed, one can expect mass ejection even from this region, although the composition ratio is dependent on the expansion velocity and the 'freezing out' density of the nucleus. Hartmann *et al.*²³ have assumed mass ejection from near the surface of a neutron star where NSE has been realized, and showed a normal abundance of iron and an

Fig. 1 Differential cosmic-ray energy spectrum of 'all particles' ($I(E)$) multiplied by $E^{2.5}$. Data (points and dash-dotted lines) are taken from refs cited in ref. 1. A straight line (P(JACEE)) denotes proton spectrum from the JACEE experiments¹³. Solid curves (Ca and Ar) illustrate the contributions of calcium and argon nuclei as deduced from the HEAO data and extrapolated by the pulsar acceleration mechanism. A sum (uppermost solid curve) of calcium and argon overabundances with $E^{-2.7}dE$ spectrum (dotted line) fits to the observed enhancement.



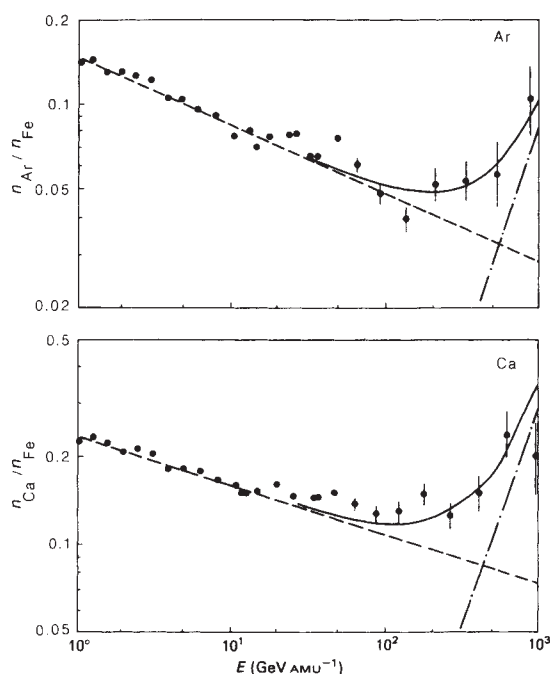


Fig. 2 Ar/Fe and Ca/Fe ratios as observed by HEAO-3C experiment¹⁷. All lines are fits to the following functions: $0.029 (E/10^3 \text{ GeV AMU}^{-1})^{-0.24} + 0.07 (E/10^3 \text{ GeV AMU}^{-1})^{1.5}$ and $0.076 (E/10^3 \text{ GeV AMU}^{-1})^{-0.126} + 0.295 (E/10^3 \text{ GeV AMU}^{-1})^{1.5}$, for Ar/Fe and Ca/Fe, respectively.

overabundance ($\sim 10^5$ solar) of neutron-rich isotopes (^{48}Ca , ^{50}Ti , ^{64}Ni , ^{66}Zn , ^{82}Se ...) at the vicinity of a neutron star. We note that this anomaly exists near the surface of the star and should not dominate if all of the SN-II ejecta are considered. It is, however, the material near the neutron star surface that will be accelerated to the highest energies.

The other model for SN-II involves more massive stars ($11 < M < 100 M_{\odot}$) and invokes a collapse of an iron core triggered by both photo-disintegration of, and electron capture on, iron group nuclei. These stars do not promptly explode via the bouncing-shock mechanism, except for those in a narrow mass range around $12 M_{\odot}$ (ref. 24), but later by neutrino heating²⁵. The resulting explosion is relatively weak and leads either to the formation of a neutron star (for $M < 20 M_{\odot}$) or a black hole ($M > 20 M_{\odot}$) depending on the mass of the remnant core. According to a nucleosynthesis calculation for a $15 M_{\odot}$ model²⁶, the $^{40}\text{Ca}/\text{Fe}$ ratio can be as large as ~ 10 times the solar value because the explosion energy is so small that only the shell material is ejected. This model also predicts a large ratio (~ 7) of S/Fe and Ar/Fe, and ~ 4 of Si/Fe relative to solar abundances. For a more massive progenitor ($25 M_{\odot}$), the $^{40}\text{Ca}/\text{Fe}$ ratio is predicted to be close to solar but here the remnant core may be too massive to remain a neutron star. Thus we associate SN-II that produce neutron stars with the calcium anomaly.

The energy spectrum resulting from supernova shock acceleration is a single power law, and its maximum energy has been estimated by Lagage and Cesarsky²⁷ at $10^{13} \text{ eV AMU}^{-1}$. Therefore, we can rule out simple supernova shock acceleration as a source of cosmic-ray high-energy enhancements. However, injected particles from a neutron star in SN-II have higher energy, because there should be about three orders of magnitude difference in the surface gravitational potential between a white dwarf (SN-I) and a neutron star (SN-II) from where the remnant is ejected. This difference, together with the strong magnetic field of a neutron star, might affect the shock wave acceleration in SN-II. Thus the high-energy feature in the cosmic-ray spectrum can be associated with SN-II and its neutron star (some entirely different origins are, of course, always possible). Fur-

thermore, if the pulsar acceleration proposed by Ostriker and Gunn^{5,6} is applied to the calcium and other nuclei formed in the SN-II, and the upper bound to the intensity deduced from HEAO satellite data is used, we find reasonable agreement between the JACEE observations and the EAS data. The observed rise of both the Ca/Fe and Ar/Fe ratios of the HEAO data (see Fig. 2) are consistent, not only with the JACEE calcium anomaly ($\text{Ca}/\text{Fe} \geq 2$; $E > 10^{14} \text{ eV}$ per nucleus), but also with the lower energy part of the pulsar acceleration ($E^{-1} \text{ dE}$) which is dominated by magnetic acceleration. Next, applying the pulsar acceleration to the calcium and argon intensities of the HEAO data, we find reasonable agreement of the enhanced 'all-particle' spectrum at 10^{14-16} eV per nucleus (see Fig. 1) with the high-energy side of pulsar component ($E^{-2.5} \text{ dE}$) which is formed during the gravitational-loss era of a pulsar. Above 10^{16} eV , cosmic rays are believed to escape from the galactic confinement and the observed steepening in Fig. 1 is thought to be a consequence²⁸. Note, however, that intensity of the nuclear components from pulsars should start their reduction rapidly above $10^{13} \text{ eV AMU}^{-1}$ due to X-ray photo-disintegration and other collision losses in the early stage of the gravitational-loss era, leaving secondary protons for further acceleration and eventual leakage from the confinement volume in the galaxy. This steepening involves various complicated parameters. Here we have used average parameters for typical pulsars²⁻⁶ and which affect slightly the low-energy slopes shown in Fig. 1. The determination of the high energy steepening will be discussed elsewhere.

Verification of our speculations requires the observation of heavy nuclei at energies above $10^{12} \text{ eV AMU}^{-1}$. For further studies, isotope identification would be especially important as one would expect an overabundance of high-energy ^{40}Ca and ^{48}Ca . (^{48}Ca is not only highly abundant in the high density NSE state but also a very stable neutron-rich nucleus.) Experimentally, mass discrimination in this energy range is difficult, but the average meson multiplicities could be used to evaluate the ratio of ^{48}Ca to ^{40}Ca (ref. 29). Other prominent overabundances we would expect are those of ^{32}S and ^{36}Ar or ^{66}Zn . ^{66}Zn is synthesized at a lower density NSE state, although its abundance in high-energy cosmic rays would be much reduced by photo-disintegration. Additional enhanced species expected would be ^{64}Ni and ^{82}Se . The observation of these specific nuclei in the high-energy cosmic-ray spectrum would be an almost conclusive signature of nucleosynthesis in SN-II and, indeed, could serve as a probe of the physical conditions in the NSE core.

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Are bipolar jets precessing?

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Whether bipolar jets from the star-forming regions precess or not is indicative of their model of origin and formation. Both the beam model¹ and the model of the shocked interaction between an isotropic stellar wind and a massive isothermal disk^{2,3} have difficulty in explaining this precession. Other models^{4–7}, which admit precession of jets, predict different periods of precession. If the recent observation of L723 (ref. 8) represents precession, then this is probably the orbital precession of a torus (disk) around a central oblate star, or the forced precession of a torus around a central star by its companion. The free precession of a central star as well as the precession of a torus may be responsible in the case of L1551 IRS5 (refs 9–11).

Astrophysical jets have been discovered in various classes of astronomical objects; in extragalactic radio sources^{12,13}, in SS433 (ref. 14), and in regions of recent star formation^{15–18}. Of these, precession of the jet flow has only been confirmed for SS433 (refs 19, 20) and for extragalactic jets^{13,21,22}. On the other hand, no explicit evidence of precession has been reported for bipolar jets from star-forming regions. However, if the basic mechanism underlying the phenomena of astrophysical jets is common (at least in part), we can also expect precession of the bipolar jets. Moreover, the discovery of, or even the absence of precession will impose restrictions on models of bipolar jets.

We can now characterize various models proposed for bipolar jets in view of the precession theory. Can they admit precession? If so, how long is a precession period?

Model A. The scaled-down version of the 'beam model' originally proposed for extragalactic double radio sources²³ has been applied to bipolar jets¹. In this model, an initially spherical flow is collimated and accelerated into the polar directions of a spheroidal gas cloud surrounding a central source like a de Laval nozzle, without the influence on the flow of the gravitational field of any objects. Thus, it seems difficult for the gaseous cloud (and confined flow) to precess due to any gravitational force because it is inconsistent with the assumption of the model.

Model B. In a similar picture, the bipolar jets are caused by the interaction between an isotropic stellar wind from a proto star and a surrounding high-density isothermal disk^{2,3}, where the shock fronts emanate into the polar directions of the disk. Because the jet caused by this mechanism is essentially non-steady, we can expect no precession.

Model C. An alternative type of model which explains collimation and acceleration of astrophysical jets is the (magneto) hydrodynamical wind-type flow in the funnel formed by a torus around a central object^{4,24–26}; the gravity of the central object has the essential role in contrast to the beam model A. In model C, the jet precession can be easily interpreted by precession of the torus. Among several ways that the torus can precess, free precession is excluded because it is difficult for the fluid torus to precess freely without damp. Also, a slaved disk model proposed for Her X-1 (ref. 27) and SS433 (ref. 28) may be inadequate for bipolar jets as rapid mass accretion from the companion cannot be expected. Hence, the remaining solutions are the forced precession of the torus by the companion and the orbital precession by the central oblate star.

The angular speed of the forced precession where the companion exerts torque on the torus²⁹ is $\Omega_p(r) = -(3/4)(G/M)^{1/2} Mm/(M+m)r^{3/2} \cos \alpha/a^3$, where M and m are respectively the mass of the central proto star and that of the companion, a the separation between them, r the distance of the torus from the central star, and α the angle between the torus equatorial plane and the binary orbital plane. Thus, when the bipolar source consists of a binary system, the possible precession period $\Pi = 2\pi/\Omega_p$ of the torus is

$$\Pi = -4.23 \times 10^2 \text{ yr } M_{10}^{1/2} m_{10}^{-1} \times (1 + m/M) a_{10\text{AU}}^3 r_{\text{AU}}^{-3/2} (\cos \alpha)^{-1} \quad (1)$$

where M_{10} and m_{10} are respectively M and m in units of 10 solar masses, $a_{10\text{AU}}$ and r_{AU} respectively a and r in units of 10 AU and 1 AU. We assume that the central proto star is a zero-age main-sequence of B type³⁰. This period depends on r and a , but typically lies within $10\text{--}10^4$ yr. Although the outer massive molecular disks were found by CS observations^{30,31}, the tidal forces by them on the inner torus is negligible.

The central star is probably deformed by its rapid rotation. If so, its oblateness also gives rise to precession of a torus orbiting it with a misaligned equatorial plane with angular speed $\Omega_p(r) = -(3/2)(R/r)^2 \Omega_r J_2 \cos \beta$, where R is the radius of the central star, $\Omega_r = (GM/r^3)^{1/2}$ the rotational angular speed of the torus, J_2 the dynamical ellipticity, and β the angle between the star's equatorial plane and the torus plane. For a model of uniform density ellipsoid, $J_2 = (2/5)(5/2)\epsilon$, where ϵ is the ellipticity of the Roche model star: $\epsilon = 0.02622 M_{10}^{-1} R_{10} V_{100}^2$. Here R_{10} is R in units of 10 solar radius and V_{100} is the equatorial rotation velocity of the star in units of 100 km s^{-1} . For other stellar models, ϵ is replaced by $f\epsilon$ with the numerical factor f of the order of unity. The period of this orbital precession is thus

$$\Pi = -2.45 \times 10^4 \text{ yr } M_{10}^{-1/2} R_{10}^{-2} r_{\text{AU}}^{7/2} (f\epsilon \cos \beta / 0.01)^{-1} \quad (2)$$

and strongly depends on r . For typical values of parameters, $\Pi = 1\text{--}10^4$ yr ($r = 0.1\text{--}1 \text{ AU}$). We emphasize that even if the central star is a single star, this precession mechanism can operate.

Model D. A final category of jet formation is due to magnetic field^{5–7,32}. In a Drain's picture⁵, the magnetic field is anchored on a proto star and is wound up by the star's rotation to make a magnetic bubble blowing off in opposite directions along the rotational axis. Therefore, when the central star precesses freely, the resultant jet also precesses with the angular speed of $\Omega_p = -\epsilon \Omega \cos \gamma$, where Ω is the star's angular speed, γ the angle between the rotational axis and the figure axis. This leads to a rather short period precession:

$$\Pi = -1.38 \text{ yr } R_{10} V_{100}^{-1} (f\epsilon \cos \gamma / 0.01)^{-1} \quad (3)$$

The problem inherent in this picture of precession is how the fluid star can precess freely.

The forced precession of the rotationally-deformed central star by a companion is also possible in the Draine's picture. The precession period is

$$\Pi = -4.84 \times 10^5 \text{ yr } m_{10}^{-1} (1 + m/M) \times R_{10}^{-1} V_{100} a_{10\text{AU}}^3 (f\epsilon \cos \delta / 0.01)^{-1} \quad (4)$$

where δ is the angle between the star's equatorial plane and the binary orbital plane. Unless the binary separation is rather small ($a \leq$ a few AU), this gives a relatively long period of precession.

Model E. In another scheme^{6,7,32,33}, the magnetic field anchored on a disk forms the bipolar jets. Hence, the precession of jets is also possible through disk precession discussed in Model C.

We next discuss the observational appearance of precession for various models. We should bear in mind that the typical age τ of CO bipolar jets is $\sim 10^4$ yr. When the precession period Π is much longer than τ , the precessional motion cannot be detected and is, therefore, less interesting; this case corresponds

to the tidal precession of the proto star by the companion [model D, equation (4)]. When $\Pi \sim \tau$ as in the cases of the tidal precession of the torus by the companion [models C, E, equation (1)] or the orbital precession of the torus around the oblate star [models C, E, equation (2)], we can observe the S-(Z-) shapes or the corkscrew pattern often found in extragalactic jets or in SS433 jets (see refs 13, 34). These two cases can be distinguished by the fact that the former needs the companion while the latter does not. Moreover, since the orbital precession of the torus is effective close to the central star [compare the r -dependence of equations (1) and (2)], it will be consistent with a narrow-collimated jet in comparison with the tidal precession of the torus by the companion. For a sufficiently short period of $\Pi \ll \tau$ as in the free precession of the central star [model D, equation (3)], the direct observation of the central source will reveal the periodic change of precession timescale Π in the luminosity or polarization. In addition, we can only observe spread jets with the opening angle not of the jet itself but of the precession angle through CO observations (and also the hollow cylindrical structure).

Finally, we try to interpret the observational data by the precessional hypothesis. The recent observation of L723 by Goldsmith *et al.*⁸ clearly shows the Z-shape structure. If this means precession, then $\Pi \sim \tau$ to produce the Z-shape. The age of L723 is estimated as $\tau = 4.7\text{--}3.0 \times 10^4$ yr. Thus appropriate models in this case are (C) or (E).

Another candidate of precession is IRS5 in L1551^{9-11,35}. In this case, the orientation of the radio contours gradually changes by about 15° from the innermost to the outermost contours¹⁰. If this indicates about one turn of the precession axis, we have the period of some 23 yr, assuming the size of IRS5 of 1.1×10^{-2} pc and the jet speed of 500 km s^{-1} . In addition to the precession of the torus [models (C) and (E)], the free precession of a central star [model D, equation (3)] may be also adequate.

Further observations involving the detection of binary nature of the central source (see ref. 11), periodic changes, and high-resolution structures would be desirable to confirm precession: the clue to astrophysical jets.

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Measurements of pH versus drop size in natural rain

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Results from numerical scavenging models¹⁻³, which estimate the acidity of precipitation falling through a polluted atmosphere, have previously been compared with pH measurements of bulk rain water or of single drops of unknown size⁴. Here we describe results from an instrument which, for the first time, allows the variation of pH with drop size to be evaluated. Field measurements show a pH minimum for drops with radii near 0.5 mm, consistent with simple mass transfer considerations.

The raindrop spectrometer uses the increase of terminal velocity with drop size to sort drops. It consists of two disks separated by a small vertical rotating shaft. The upper disk has a small sector-shaped aperture through which rain passes, and the lower disk is divided into sectors by radial walls so that water is collected. Because the disks rotate at the same rate, larger drops (falling at higher speeds) intersect the lower disk more nearly beneath the aperture than do smaller drops (see Fig. 1).

The theory, design and testing of the instrument are described in detail in ref. 5. It comprises two perspex disks of 0.25 m radius, separated vertically by 0.5 m, and rotating at 300 r.p.m. By choosing a 20° aperture width, 1-ml samples can usually be obtained in a few minutes. However, a drop of given size may land within a 20° area on the lower disk, depending on where it entered the aperture; therefore, collecting sectors will contain water from overlapping ranges of drop size.

By computing drop trajectories, collection efficiencies are derived for each sector as a function of drop size and wind speed. These collection efficiencies relate the volume and pH of water collected in each sector to droplet concentration and pH in each drop-radius range. Droplet concentrations and pH spectra are obtained from measured volumes and pH in each sector by a least-squares method (six drop-radius ranges and seven sectors). When there is zero horizontal wind, samples in each collecting sector are nearly independent (Fig. 2a). However, a wind speed of 1 m s^{-1} produces a bimodal collection efficiency for each sector because drops fall further from the aperture when the aperture is moving upwind, and closer to the aperture when the aperture is moving downwind. As shown in Fig. 2b, this affects the ability of the instrument to resolve collected water into drop-size classes. By placing a semicircular mask over the top of the spectrometer and aligning the straight edge with the wind, the collection efficiency resolution is much improved (Fig. 2c) and sensible results should be obtainable in winds of 3 m s^{-1} .

Tests carried out to validate the theoretical description of the instrument are summarized in Table 1. Tests 1, 5 and 6 show

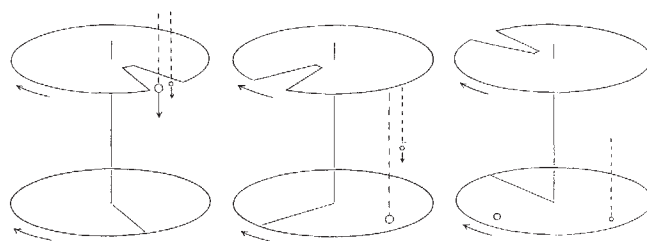


Fig. 1 Schematic of spectrometer operation. The larger drop falls faster, intersecting the lower disk before the smaller drop. The disk rotates during the intervening time, collecting the two drops at different sites.

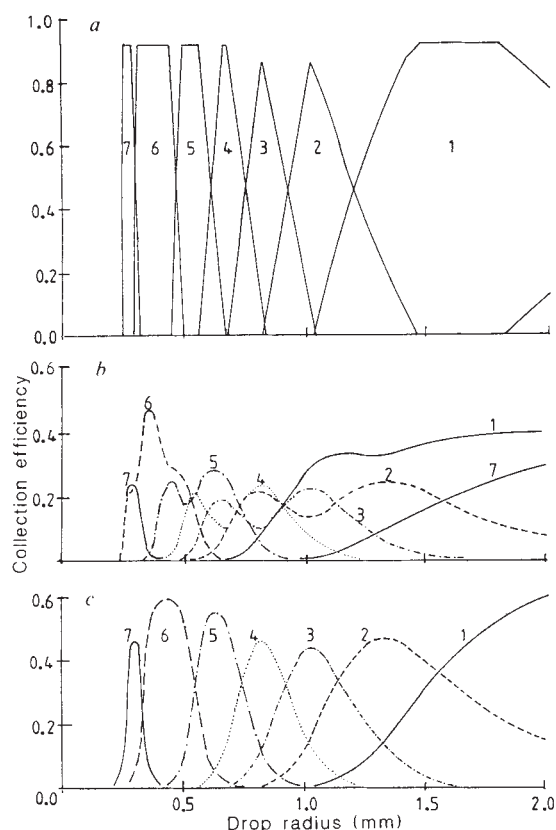


Fig. 2 Collection efficiencies of sectors 1-7 as a function of drop radius: *a*, zero wind speed; *b*, wind speed = 1 m s^{-1} ; *c*, wind speed = 1 m s^{-1} but with a semicircular mask placed over the spectrometer.

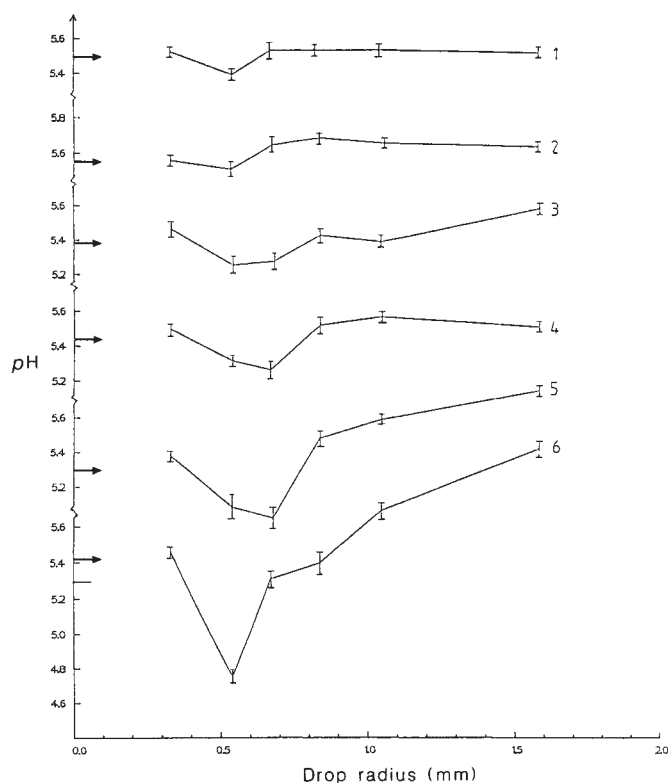


Fig. 3 pH versus raindrop size. Bulk-water pH values are indicated by arrows at the left.

that drops are collected as predicted, and tests 2 and 3 check that there is no bias in derived drop distributions. Test 4 demonstrates that water is not chemically contaminated by the collection process. Errors in the derived pH spectra are therefore almost entirely due to uncertainties in attributing water collected in a sector to drop-size ranges. These errors are estimated as part of the least-squares procedure and include the effect of wind through the collection efficiency matrix.

Figure 3 shows preliminary pH spectra from measurements of natural rain; ancillary data are given in Table 2. All spectra exhibit a pH minimum near a drop radius of 0.5 mm. The independently measured bulk-water pH values, shown by arrows at the left of Fig. 3, are lower than the CO_2 equilibrium values. Rain-bearing systems approaching Auckland are predominantly of maritime origin and will have had little contact with polluted airmasses; in such cases we may assume a flat in-cloud pH spectrum, with changes being due to sub-cloud scavenging. The results shown in Fig. 3 suggest that drops with radii near 0.5 mm

scavenge more efficiently. The observed spectrum shape is similar to that predicted in detailed models^{1,3} for low NH_3 concentrations, but can be interpreted in a simple way as follows: The rate of change of dissolved gas concentration is proportional to both the mass transfer coefficient, k (a function of diffusion coefficient, Reynolds number and Schmidt number), and the surface-area-to-volume ratio, β . For small drops both factors are high and rapid gaseous absorption results. As k and β initially decrease with increasing drop size⁶, pH should increase rapidly with size for small drops. However, the mass transfer coefficient has a minimum for intermediate drop radii, so that a pH maximum results at about 0.1–0.4 mm radius. Thereafter, k increases and β decreases, leading to the observed minimum pH near 0.5 mm radius.

Table 2 shows a correlation between both spectrum variance and bulk pH, and time since rain shower onset. Only spectra 4 and 6 are directly comparable, having been sampled from the same rainfall system, but the similar bulk pH values suggest that

Table 1 Tests of spectrometer performance

Test no.	Quantity tested	Associated error	Comments
1	Angular capture range	$\pm 3^\circ$	Individual drops in a stream were sized and fall speeds measured before landing on dye-coated paper in each sector.
2	Loss of water	3% volume	Measured quantities of water poured into sectors compared with quantities after spectrometer had been run. Losses are consistent with evaporation into dry laboratory air.
3	Splashing	$\pm 0.2\%$ volume	Most splash products fall into the same sector as the parent drop.
4	Contamination	± 0.02 pH units	pH measured before and after passing water through the instrument.
5	Drop-size distribution	$\pm 20\%$	Comparison of distributions derived from dyed filter paper and from the spectrometer, both exposed to rain. Errors were within limits expected from filter-paper counts.
6	Angular capture range	—	The spectrometer was operated in rain with dyed filter paper in each sector. Drops were all within expected sectors (apart from obvious splash products).

Table 2 Data relevant to pH spectra in Fig. 3

Spectrum	Wind speed (m s ⁻¹)	Rainfall rate* (mm h ⁻¹)	Start† (min)	Duration (min)	pH variation‡	Bulk pH
1	1.1 ± 0.2	4 ± 0.5	40	40	0.06	5.50 ± 0.02
2	0.5 ± 0.2	7 ± 0.5	30	30	0.07	5.54 ± 0.02
3	1.6 ± 0.3	5 ± 0.5	20	30	0.12	5.38 ± 0.02
4	1.4 ± 0.2	5 ± 0.5	24	35	0.13	5.44 ± 0.02
5	0.7 ± 0.3	7 ± 0.5	0	22	0.29	5.30 ± 0.02
6	1.9 ± 0.3	8 ± 0.5	0	24	0.43	5.30 ± 0.02

* Rainfall rate derived from water volume collected by spectrometer.

† Time of start of sampling relative to start of rain shower.

‡ Standard deviation computed from the six pH spectral points in each spectrum.

all airmasses were comparable. These data suggest that the time constant for spectrum change is ~20 min.

These results are the first test of the drop-size dependence predicted by acid-rain scavenging models; laboratory wind-tunnel experiments are not guaranteed to simulate natural conditions and bulk-water measurements do not allow detailed microphysical inferences. For example, the maritime airmass examples all have comparable bulk-water pH values near the CO₂ equilibrium, but when size-fractionated results are plotted, pH varies by as much as 1.2 units.

The observed transient pH spectra agree qualitatively with present steady-state raindrop scavenging models assuming low

NH₃ concentrations^{1,3}. There is, however, a need for comparison between time-dependent models and data such as our instrument can obtain. Collection of rain over a wider range of conditions, with a more comprehensive error treatment and chemical analysis of other components, is also desirable to allow closer comparison between theory and measurement.

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Palaeolatitude drift history of displaced terranes in southern and Baja California

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The delineation of microplate-tectonic terranes¹ which make up western North America, the timing of their accretions to western North America and the identification of their original geological settings are all topics of debate^{2,3}. The most recent addition to this family of terranes is the Santa Lucia/Orocopia allochthon^{4,5} (SLOA) (Fig. 1), which is made up of Salinia^{6,7} and several other smaller terranes⁸ and is located in central and southern California. This composite terrane amalgamated in the Cretaceous and accreted to southern California by the early Palaeogene^{4,5}. We describe here new palaeomagnetic results from localities outboard from the SLOA in southern California (USA) and Baja California (Mexico). These results are used to define a newly identified allochthonous terrane in southern and Baja California (Peninsular Ranges terrane⁹, PRT) which is outboard from the SLOA, estimate the PRT's palaeolatitude drift history for the past 160 Myr, and infer its original geological setting. PRT and SLOA palaeolatitude drift histories are then combined to produce a new perspective on the tectonic development of southern and Baja California over the past 160 Myr.

Our palaeomagnetic results are based on samples collected from Palaeocene (Silverado Formation), Cretaceous (Ladd and Williams Formations) and Jurassic (Bedford Canyon Formation) sedimentary rocks of the Peninsular Ranges (southern California), and Cretaceous sedimentary rocks of the Santa Monica Mountains (southern California; Tuna Canyon Formation) and El Rosario (Baja California; Punta Baja and Rosario Formations). At each sampling locality, four oriented specimens were collected from each of several sites. The Cretaceous sampling localities and their relations to our proposed terrane boundaries are shown in Fig. 1 (see also Table 1).

All specimens were thermally demagnetized at 50 or 100 °C steps up to at least 500 °C. Zijderveldt diagrams (Fig. 2a) were used to estimate the characteristic remanences associated with

the primary and any secondary magnetizations. Secondary magnetizations were common and easily removed by 200 °C. Characteristic remanences associated with the primary magnetization (that is, that remanence acquired at the time of initial sediment deposition) were recovered routinely between 200 and 500 °C. Rock magnetic analysis indicated that the primary magnetization was carried by detrital magnetite. If good characteristic remanences could not be discerned, the specimens were discarded from further analyses. Three criteria were then applied to the characteristic remanences to further establish that they were primary in origin. The criteria (see Table 1) were: (1) the presence of well-defined (antipodal polarity) reversals; (2) the passage of a fold test, and (3) *in situ* directions significantly different from the present axial-dipole field. At each of the sampling localities, either two or three of the criteria were satisfied. The results from the Palaeocene and Jurassic rocks of the Santa Ana Mountains should be considered more tentative than the Cretaceous results due to the smaller number of sampling sites at these two localities.

The resultant field vectors for each locality, before (*in situ*) and after bedding correction, are plotted in Fig. 2b, and listed in Table 1. The apparent similarity of most *in situ* average field vectors could be interpreted to mean that the rocks were remagnetized after folding while still at lower latitudes. However, the presence of well-defined intervals of reversed and normal polarity in all localities, the fact that the magnetostratigraphies correlate well with the expected magnetic polarity stratigraphy estimated from known biostratigraphical data¹⁰, and the fact that three out of five localities clearly pass fold tests argue convincingly against such a possibility. More detailed analyses of the basic data are presented elsewhere (ref. 10 and L.K.M. and J. G. Fry, unpublished data).

The inclination results were used to estimate palaeolatitudes for each locality based on an axial-dipole mapping. Each locality's palaeolatitude was then normalized to its current latitude to produce the quantity $\Delta\theta$ (palaeolatitude minus present-day latitude) (Table 1).

Our normalized palaeolatitudes, $\Delta\theta$ s, and their associated declinations are compared with other previously published results^{5,7,8,11-13} of Cretaceous age in Fig. 1. This shows that all localities south of the Oak Ridge Fault (ORF) and its extension into Santa Barbara Basin have small negative $\Delta\theta$ s of about -6°.

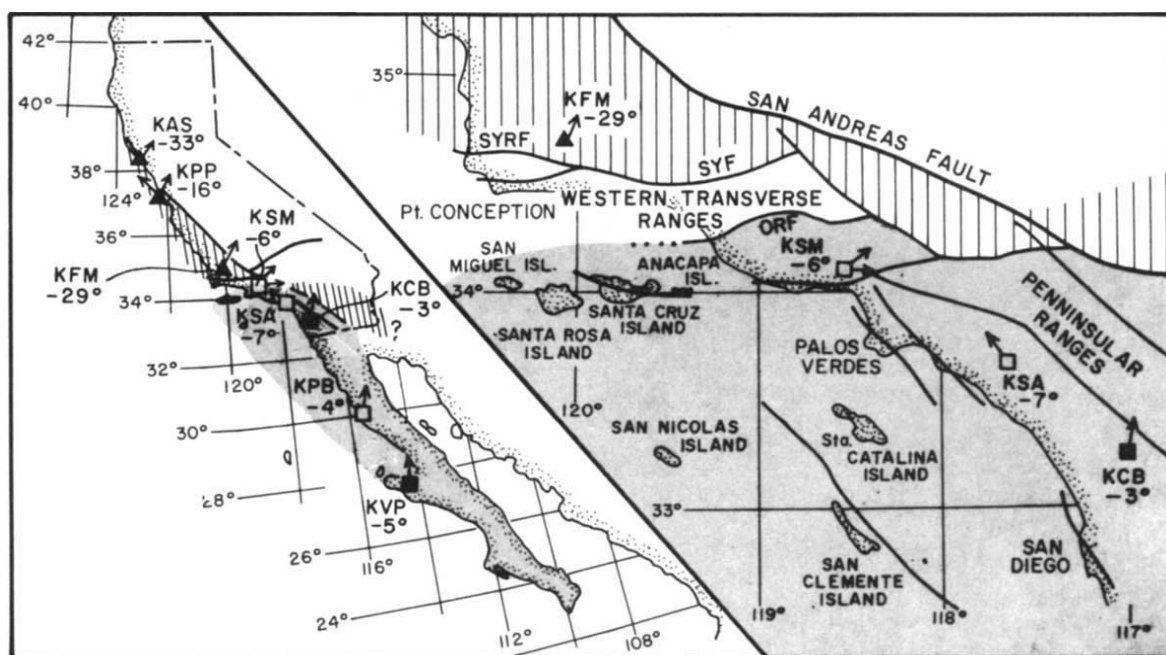


Fig. 1 Cretaceous normalized palaeolatitudes, $\Delta\theta_s$, of rocks from southern and Baja California (see Table 1). $\square$, Results from this study; $\blacksquare$ and $\blacktriangle$, previously published results (KPP⁵, KFM⁷, KAS⁸, KCB¹¹, KVP¹²). $\square$, $\blacksquare$, Results from the PRT; $\blacktriangle$, results from the SLOA. Arrows indicate directions of tectonic-corrected declinations from each locality. The similarity of $\Delta\theta_s$ south of the Oak Ridge Fault (ORF) and its extension into the Santa Barbara Basin suggest that this region, called the Peninsular Ranges terrane (shading), was spatially coherent in Cretaceous time. The Santa Lucia-Orocopia allochthon is shown by the vertical-lined pattern. Its southern boundary in the Western Transverse Ranges is probably the Santa Ynez (SYF) and Santa Ynez River (SYRF) faults. The normalized palaeolatitudes are also plotted against time in Fig. 3.

Table 1 Summary of palaeomagnetic results

Locality name*	KSM	KPB	KSA	JSA	PSA
Locality lat. (°N)	34.1	30.0	33.7	33.7	33.7
Locality long. (°E)	241.4	244.3	242.3	242.3	242.3
Age†	Tur/Maas.	Camp/Maas.	Tur./Camp.	Jurassic	Palaeocene
Confidence criteria‡	A, B, C	A, B	A, B	A, C	A, C
No. of sites§	6, 6	17	20	4	4
In situ inclination	37.2, 35.5	46.2	44.6	28.1	45.2
In situ declination	26.4, 104.8	-5.2	5.7	16.6	23.4
In situ α_{95}	9.9, 10.2	13.5	9.2	20.5	9.4
Corrected inclination	45.8, 48.0	44.5	45.1	11.0	43.2
Corrected declination	55.5, 90.8	3.5	-36.4	25.7	-26.0
Corrected α_{95}	7.0, 5.8	8.2	6.7	12.1	8.8
Palaeolatitude¶	28.0 ± 5.0	26.2 ± 6.5	26.6 ± 5.4	5.6 ± 6.2	25.1 ± 6.8
$\Delta\theta$ ¶	-6.1 ± 5.0	-3.8 ± 6.5	-7.1 ± 5.4	-28.1 ± 6.2	-8.6 ± 6.8

* KSM, Cretaceous Tuna Canyon Formation-Santa Monica Mountains; KPB, Cretaceous Punta Baja and Rosario Formations-El Rosario, Baja California; KSA, Cretaceous Ladd and Williams Formations; JSA, Jurassic Bedford Canyon Formation; and PSA, Palaeocene Silverado Formation—all from the Santa Ana Mountains. KSM, KPB and KSA sampling localities are shown in Fig. 1. JSA and PSA sampling localities are within 10 km of KSA.

† Tur., Turonian; Camp., Campanian, Maas., Maastrichtian; all late Cretaceous stages.

‡ A, Presence of reversals with antipodal polarity, B, passage of fold test, C, *in situ* direction significantly different from the present day field.

§ Four specimens were averaged per site.

|| Two localities, each with six sites, located on opposite flanks of a large faulted anticlinal structure. Both localities have antipodal reversed sites. Agreement between two localities is markedly improved when corrected for tectonic folding (passes fold test) and declinations agree with previous estimates of large-scale rotation of the Santa Monica Mountains¹⁵, but declinations suggest some residual differential rotation between the two localities.

¶ ± Indicates 95% confidence interval.

These results indicate that this region was about 6° farther south during late Cretaceous time relative to its present geographical position. This region, which has been termed the Peninsular Ranges terrane⁹ (PRT) based on geological evidence^{9,14}, includes the Simi Hills, Santa Monica Mountains, Peninsular Ranges, North Channel Islands and Baja California. The SLOA north of the Santa Ynez Fault (SYF) has much larger negative $\Delta\theta_s$ which indicate that it was even farther south during the late Cretaceous. The region between the Oak Ridge Fault and

the Santa Ynez Fault (SYF) and its extension, the Santa Ynez River Fault (SYRF), has questionable affinities.

In contrast to the negative $\Delta\theta_s$ of the PRT and SLOA, the $\Delta\theta$ for the North American craton was +9° during the late Cretaceous¹⁵, indicating that the craton was about 9° farther north during this time relative to its present geographical position (Fig. 3). The 95% confidence interval ($\pm 2\sigma$) about the stable craton $\Delta\theta$ estimate for any time interval is $\pm 8.6^\circ$ (ref. 16) (Fig. 3). The PRT and SLOA $\Delta\theta_s$ are significantly different from the

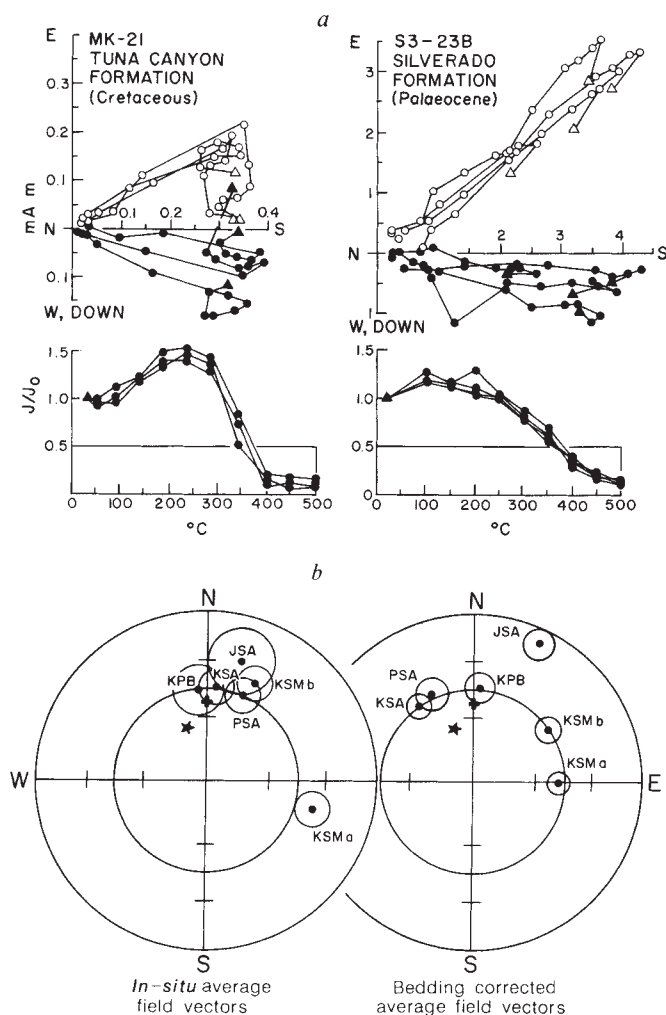


Fig. 2 Zijderveld diagrams and stereoplots of some results from this study. *a*, Typical Zijderveld diagrams for two sites of reversed polarity (Upper Cretaceous Tuna Canyon Formation, Palaeocene Silverado Formation) showing intensity loss and vector change with stepwise heating to 500 °C. The filled (open) circles represent the tips of NRM vectors after each heating step plotted in the horizontal (vertical) north, east (north, down) plane. *b*, *In situ* and bedding-corrected average field vectors of the localities listed in Table 1. The present axial-dipole field vector is represented by a cross. The expected late Cretaceous field vector is represented by a star. KSMa and KSMb are each the average of six sites on opposite sides of a large-anticlinal structure in the Santa Monica Mountains.

stable craton $\Delta\theta$, for they differ by -15° and more than -23° respectively from the stable craton result.

The distinctly different $\Delta\theta$ s of the PRT, SLOA and North American stable craton indicate that they were all farther separated in latitude during the late Cretaceous than their present relative geographical positions might indicate. Less uniquely, the similarity of $\Delta\theta$ s within the PRT indicate that it has remained spatially coherent since at least the late Cretaceous, with only minor re-ordering in the late Neogene due to extensional tectonics. This late Neogene tectonic regime has caused a large clockwise rotation of the Santa Monica Mountains^{3,17} as noted by the rotated declinations of locality KSM (Fig. 1).

The Cretaceous $\Delta\theta$ s and other Mesozoic and Cenozoic age $\Delta\theta$ s (refs 5, 8, 13, 17–19) from these terranes are plotted against time in Fig. 3 to estimate each terrane's drift in palaeolatitude over the past 160 Myr. The normalized palaeolatitude, $\Delta\theta$, drift curve for the North American stable craton¹⁵ is plotted for reference. We can see that all the $\Delta\theta$ s from the PRT (squares),

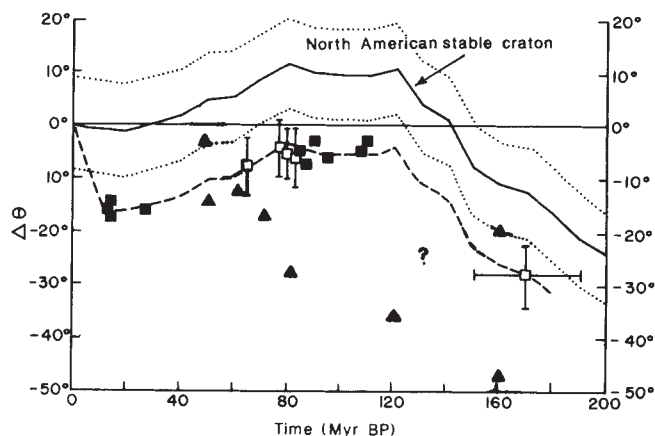


Fig. 3 Normalized palaeolatitudes of the SLOA (triangles) and PRT (squares) plotted against time. The open symbols (with 95% confidence intervals) are results from the present study, the closed symbols are previously published results^{5,7,8,11–13,17–19}. The North American stable craton reference curve was derived from Irving and Irving¹⁵ and the $\Delta\theta$ curve for the SLOA (triangles, shading) is from ref. 7. The dotted lines on either side of the stable craton reference curve indicate the 95% confidence interval ($\pm 2\sigma$) about this curve.

all older than 15 Myr, are systematically lower than the stable-craton curve by $\sim 15^\circ$. Shifting the stable-craton curve downward 15° produces the dashed curve in Fig. 3 which not only fits all the PRT data but also connects data of different ages from within the PRT. The 95% confidence interval about the 15° offset of the PRT $\Delta\theta$ curve (dashed curve in Fig. 3) is $\pm 6.4^\circ$, indicating that the average $\Delta\theta$ s of the stable-craton and the PRT were different by more than 4σ before 15 Myr ago. The remarkable match between the offset stable-craton reference curve and these results suggests that this terrane has drifted as part of North America for most of its geological history, but that during this time it was located about 15° lower in latitude relative to the stable craton. This interpretation places the PRT along the western margin of Central America between 15 and 160 Myr ago. The PRT must have moved northward 15° since ~ 15 Myr ago to reach its present position relative to the stable craton.

The $\Delta\theta$ s of the SLOA, on the other hand, are much lower than the PRT results, and their drift pattern^{7,8} is significantly different in shape from the stable-craton reference curve. This indicates that the SLOA was not attached to the North American plate until it accreted in early Palaeogene time.

A simple history of these two terranes for the past 160 Myr can be summarized as follows: (1) location of the PRT in Central America as part of the stable craton from 160–15 Myr ago; (2) internal amalgamation of the SLOA in Cretaceous time at lower palaeolatitudes; (3) subsequent drift of the SLOA northward past and outboard from the PRT; (4) accretion of the SLOA with southern California in early Palaeogene time; (5) northward movement of the PRT along the cratonal margin since 15 Myr ago; (6) accretion of this terrane with the SLOA and North American stable craton before 5 Myr ago, (7) continued strike-slip motion along southern California faults in late Neogene time, producing the dispersed terrane pattern that we see today. The SLOA/PRT suture zone is expressed tectonically by the Transverse Ranges of southern California, and recent geophysical studies²⁰ support this hypothesis. The most notable elements of this history are the location of the PRT in Central America as part of the stable craton until 15 Myr ago, and its subsequent northward displacement in Neogene time. This has never been taken into account in Cenozoic tectonic reconstructions of southern and Baja California^{3,21}.

These results present a new view of the tectonic history of southern and Baja California and explain several perplexing aspects of the Neogene tectonic development of this region. The

results, however, are inconsistent with some other tectonic reconstructions²¹ and require further study of their possible implications.

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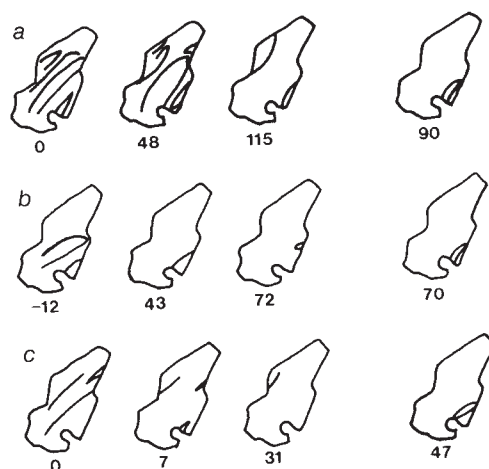


Fig. 1 Domain observations during hysteresis at room temperature (a), 50 °C (b) and 80 °C (c). Numbers refer to magnetic field strengths (in oersteds); positive fields point downwards. At room temperature (a), the particle is nearly saturated by 115 Oe and fully saturated at 193 Oe. At 50 °C (b), a state of near saturation occurs at 72 Oe, with full saturation at 114 Oe. At 80 °C (c), near saturation occurs at 31 Oe, with total saturation at 72 Oe. The final figure in each set shows the first domain pattern observed during ramp-down of the field, and the field value at which it occurred. As temperature increases there is a decrease in the external field values required for saturation and loss of saturation. The particle size is $\sim 35 \times 70 \mu\text{m}$.

Domain observations of titanomagnetites from room temperature to Curie point and the nature of thermo-remanent magnetism in fine particles

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Single-domain theory¹ has been remarkably successful as a theoretical basis for rock magnetism and hence palaeomagnetism. However, the grain size of much of the magnetic material present in rocks is too large to be single-domain according to classical theory². The resolution of this paradox may lie in the recognition that difficulties in domain wall nucleation³⁻¹⁰ can preclude the multi-domain state even though it is energetically favoured. Thus, multi-domain particles are observed in a single-domain state in rocks carrying saturation remanence magnetization (IRMs). Inhibition of domain wall nucleation has also been shown to produce high-coercivity materials suitable for permanent magnets¹¹⁻¹³. Thermo-remanent magnetization (TRM) is one of the most important mechanisms of remanent magnetization for the study of palaeomagnetism, being acquired for example by lavas as they cool on the Earth's surface. We present here domain studies of titanomagnetites showing the increasing importance of the magnetostatic energy in the external field as temperature is increased, and that rocks carrying weak-field TRM, like IRMs, contain multi-domain grains in a single-domain state. These particles contribute very large moments to TRM; indeed, particles of this nature may carry a substantial part of the palaeomagnetic signal. The results also suggest that weak-field cooling of dispersions of suitable fine particles might leave them in a particularly hard magnetic state of some utility.

Domain patterns in titanomagnetites of titanium content $\chi = 0.6$ were investigated during hysteresis at different temperatures

and during weak-field thermal cycling, to investigate the role of domain wall nucleation in TRM. The titanomagnetites studied were from DSDP (Deep Sea Drilling Projects) basalts from Leg 49¹⁴, and were mechanically and ionically¹⁵ polished to remove surface stress. The Bitter pattern technique¹⁶ was employed to observe the domain structure, using a special high-temperature colloid. The samples were placed in a brass heating stage and temperature was measured with a thermocouple. The stage was placed between the poles of an electromagnet, so that hysteresis loops could be observed, as well as thermal cycling in a variety of fields. A video camera attached to the microscope, coupled with a video recorder, allowed continuous recording of domain movement and configuration throughout each experiment.

Figure 1 shows the domain patterns resulting during hysteresis at room temperature (a), 50 °C (b) and 80 °C (c). Note the systematic decrease in the field required for saturation, as the temperature increases. Note also that with increasing temperature, the particle remains saturated at lower field strengths during ramp-down of the field. Figures 2 and 3 show heating and cooling cycles of the same particle in external fields of 1 Oe and 45 Oe, respectively. The particle saturates at lower temperature in the higher field. Both cycles carried the particle through its Curie point of 150 °C.

The observed domain patterns attest to the increasing dominance, at higher temperature, of the magnetostatic energy, $M_s H$, where M_s is the saturation magnetization and H the external field. This is to be expected, as other terms are dependent on M_s to higher powers (for example, the demagnetization energy is proportional to M_s^2). As M_s decreases with increasing temperature, the linear term becomes dominant.

No substantial increase in the number of domain walls is seen with increasing temperature. This possibility had been considered because the temperature dependence of the various energy terms should allow nucleation to occur more easily. Neither in these samples, nor in larger particles with numerous walls, have we seen a substantial increase in the number of walls as the sample was heated. This is again consistent with the increasing importance of the magnetostatic energy in the external field relative to the demagnetizing energy.

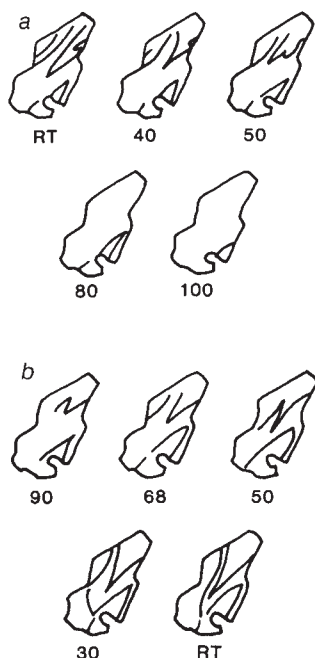


Fig. 2 Domain states during heating (*a*) and cooling (*b*) in the geomagnetic field (1 Oe). Numbers refer to temperature (°C); RT, room temperature. Note the decrease in the number of walls as temperature increases (*a*). A state of near saturation exists at 100 °C, with no domain pattern evident at 120 °C. During cooling (*b*), a faint first wall appears at 110 °C, with definite structure by 90 °C. Again, fewer walls are seen at high temperature.

It has been shown⁵⁻¹¹ that particles larger by orders of magnitude than the classically predicted single-domain size may be found in a single-domain state in saturation remanence. Such a particle has a relatively large moment and hence could be of importance in palaeomagnetic studies if it behaved similarly in TRM. We selected three particles that were able to remain in a saturated or nearly saturated state while in saturation remanence, and subjected them to thermal cycling in the Earth's field. Each particle was demagnetized in an alternating field (a.f.) to 200 Oe before heating, to ensure that the particle was initially in a multi-domain state. On cooling, the first particle showed no domain pattern; on a.f.-demagnetization to 50 Oe a pattern became apparent. Figure 4 shows the second particle before heating (*a*), after cooling in the Earth's field (*b*) and after cooling in a field of 30 Oe (*c*). A nearly saturated state results after cooling in the Earth's field, and the particle is saturated after cooling in the 30-Oe field. The application of a 50-Oe a.f. demagnetization, following cooling in the 30-Oe field, gives rise to the pattern shown in Fig. 4*d*. A third particle also showed a nearly saturated state after cooling in the Earth's field. Grains of the order of tens of micrometres are thus capable of behaving as single-domain particles in weak-field TRM. These particles have large magnetic moments, yet would follow Néel's $\tanh H$ field dependence¹.

The results reported here suggest that TRM in small particles is partially controlled by nucleation. As nucleation is grain-size dependent, such TRM should have a similar grain-size dependence. In an assemblage of small particles ranging from true single-domain to true multi-domain, there would be three contributions to the final TRM. First, there will be a single-domain contribution, which obeys Néel's theory. Second, there will be a contribution from particles in a multi-domain state¹⁷. Note that the multi-domain patterns commonly observed in titanomagnetites are not fully understood. It has been suggested that particularly complicated, finely convoluted patterns are stress-controlled and carry stable TRM¹⁸, but this remains controversial. Third, there will be a contribution from multi-domain

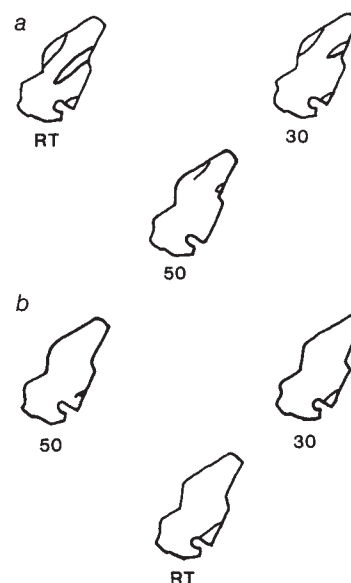


Fig. 3 Domain states during heating (*a*) and cooling (*b*) in an external field of 45 Oe. During heating (*a*), the size of reverse domains decreases rapidly, so that by 50 °C only one reverse domain is left. The particle is saturated by 70 °C. During cooling (*b*), no reverse domains are nucleated until 55 °C, a much lower temperature than in the low-field case (Fig. 2).

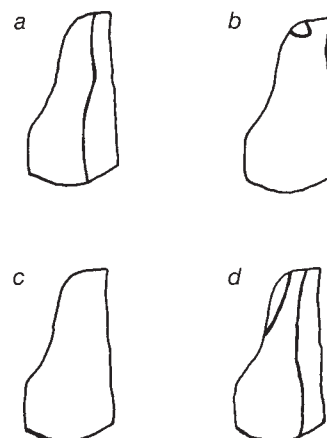


Fig. 4 Domain states of a $14 \times 7 \mu\text{m}$ titanomagnetite particle. *a*, Before heating in the Earth's field, after a 200 Oe a.f. demagnetization; *b*, After cooling from the Curie point. Note the nearly saturated state of the particle. Another particle of comparable size remained in a single-domain state after a similar experiment. *c*, After cooling from the Curie point in an external field of 30 Oe. Notice the saturated state of the particle. *d*, After application of a 50 Oe a.f. demagnetization.

grains which have failed to nucleate walls. This TRM should unblock at temperatures near to the Curie point and behave as do large single-domain particles. Stability to a.f.-demagnetization will depend on the nucleation fields of the particular grains, giving a distribution of the microscopic coercivities.

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Magnetotactic bacteria and single-domain magnetite in hemipelagic sediments

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Remanent magnetism in marine sediments has been used extensively over the past twenty years to calibrate the geological time-scale, study geomagnetic reversals and secular variation, and measure the rates of seafloor spreading. Although these sediments may contain different magnetic minerals, magnetite is the most commonly observed and magnetically stable phase, and its size, shape and post-depositional fate affect the magnetic remanence of the sediments. Biogenic magnetites are single-domain, with a high natural magnetic remanence (NRM), and have been suggested as a significant source of magnetic remanence in marine sediments. We have studied surface sediments from the Santa Barbara Basin and report the occurrence of living magnetotactic bacteria and the deposition of biogenic ultra-fine-grained, single-domain magnetite. Using a novel extraction technique, transmission electron microscopy and SQUID magnetometry, we show that these bacteria and the magnetite they produce are the major source of stable remanent magnetism in these sediments.

Studies of hemipelagic sediments from several localities in the eastern Pacific show a maximum intensity of magnetic remanence occurring in the surface sediments^{1,2}. Rock magnetic studies indicate a dramatic decrease of intensity with depth, correlated with a loss of the fine-grained component^{3,4}. However, the limited resolution of the techniques used has so far prevented the specification of the morphology of the fine-grained magnetite, or its mechanism of formation⁵. The high intensity of natural magnetic remanence and bacterial activity in the surface sediments suggested biogenic magnetite as a probable source of the sub-micrometre-sized magnetite.

Our samples were collected from three different sites in the Santa Barbara Basin (34° 14' N, 120° 1' W), at a depth of 598 m and a temperature of 8 °C. The top 3 cm of sediment was removed from box cores and either fixed with 2.5% glutaraldehyde immediately after collection or left untreated. All the samples were kept at 8 °C.

We examined the unfixed sediment with a low-power (×160) light microscope by placing a drop of sediment on a glass slide. When the south end of a stirring bar magnet was placed next to the drop, magnetotactic bacteria present in the sediment accumulated at the edge of the drop. To study these bacteria and the ultra-fine-grained magnetite they produce by transmission electron microscopy, we devised a novel extraction technique. Carbon-coated copper grids were placed face down on drops of sediment suspension and the south pole of a cobalt rare-earth magnet was suspended 1 cm above the drops, for

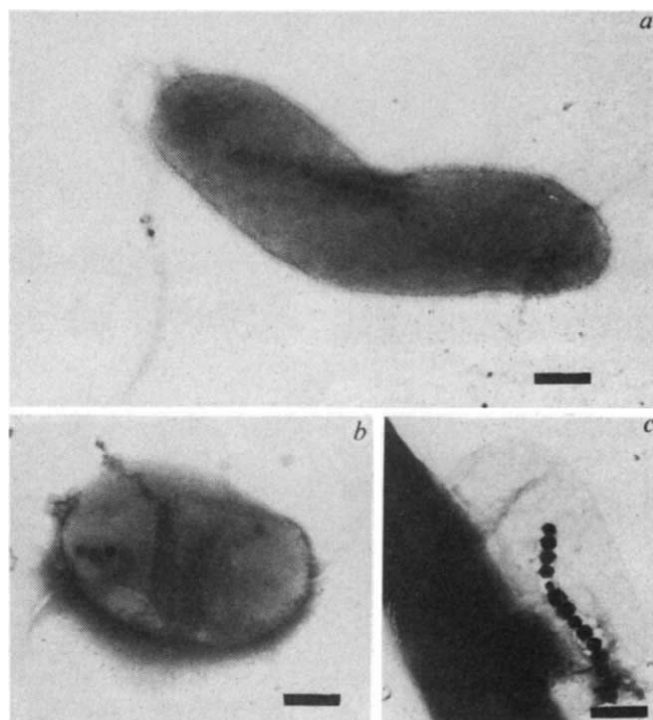


Fig. 1 Transmission electron micrographs of magnetotactic bacteria from the Santa Barbara Basin; all scale bars 200 nm. *a*, *Vibrio* with chain of 11 crystals of magnetite and one polar flagellum. Note the irregular shape of the crystals. *b*, Coccoid with chain of four crystals. *c*, Rod with chain of 13 crystals.

20–30 min. Some grids were treated with 1% uranyl acetate for 1 min to stain the bacteria. The grids were observed with a Phillips 201 transmission electron microscope at 80 kV. Identification of the crystals as magnetite was made by electron diffraction^{5,6}.

We observed several morphological types of magnetotactic bacteria in preparations of unfixed sediment samples (Fig. 1). *Vibrio* and rod-shaped bacteria were seen (Fig. 1*a, c*), but the most common morphology was coccoid (Fig. 1*b*). In each case, the crystals of magnetite were arranged in a chain 10–20 crystals long. The individual crystals were between 40 and 60 nm in size and cuboidal, rectangular or irregular in shape. The sizes of all the well-formed crystals fall within the calculated stability field for single-domain magnetite⁷; in fact, to date, all reported sizes of bacterial magnetites fall within this range^{8–10}. The irregular crystals may be produced by the dissolution of well-formed crystals (H. A. Lowenstam, personal communication).

Aggregates and chains of ultra-fine-grained magnetite free of bacteria were seen in both unfixed (Fig. 2*a*) and fixed (Fig. 2*b, c*) material. Like the magnetite seen in the bacteria, most of the crystals were 40–60 nm in size and cuboidal, rectangular or irregular in shape. In one instance an intact magnetosome was observed (Fig. 2*c*). The gradual increase in crystal size along the chain, with a few much smaller crystals at one end, is characteristic of magnetosomes in many magnetotactic bacteria. Crystals which were roughly teardrop-shaped and larger in size (100–120 nm) were also seen (Fig. 2*d*).

The magnetic properties of the sediment were analysed using saturation isothermal remanent magnetization (sIRM) and alternating-field demagnetization (AF) with a SQUID magnetometer¹¹. Both fixed and unfixed sediments were examined, as 0.1-g aliquots in quartz containers. The coercivity spectrum generated (squares in Fig. 3) indicates that the primary remanence carrier is single-domain magnetite. In a previous study, the coercivity spectrum of a marine magnetotactic bac-

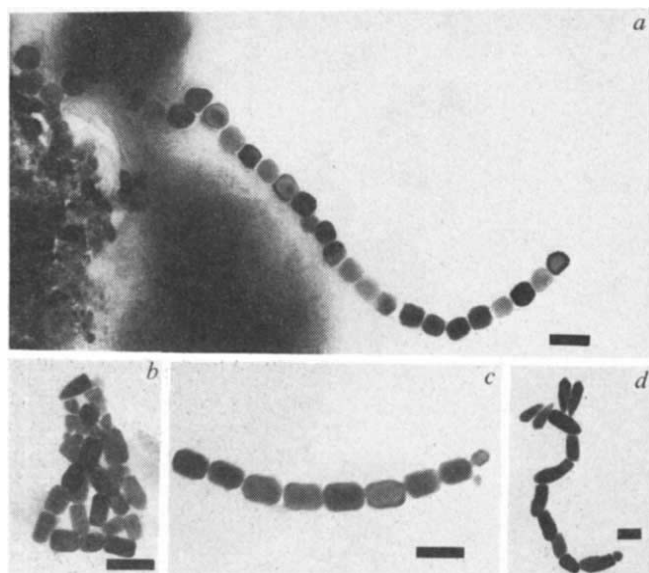


Fig. 2 Transmission electron micrographs of ultra-fine-grained magnetite from the Santa Barbara Basin. All but *a* were from fixed material; all scale bars 100 nm. *a*, Chain of 30 cuboidal crystals extracted from unfixed material. *b*, Cluster of prismatic and irregular magnetite crystals. *c*, Chain of crystals which appears to be an intact magnetosome. Note the smaller crystal at the right-hand terminus. *d*, Chain of irregular and teardrop-shaped crystals.

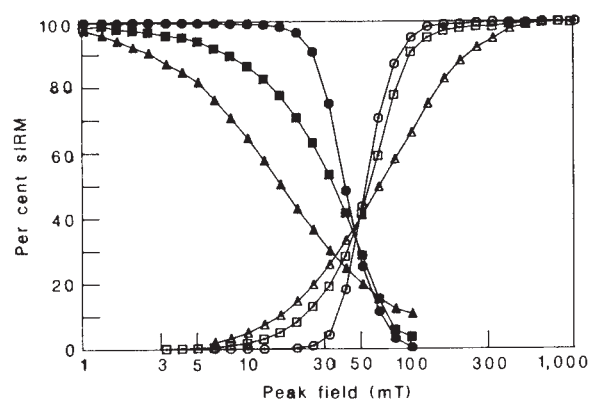


Fig. 3 Coercivity spectra of Santa Barbara Basin surface sediment (squares), pellet of marine magnetotactic bacteria from Laguna Figueroa, Baja California, Mexico¹² (circles) and beach sand with 10% w/v content of coarse-grained magnetite from Laguna Figueroa¹² (triangles). Open symbols, IRM; solid symbols, AF demagnetization.

terium was determined (circles, Fig. 3)¹². Collected from a salt-water marsh, these bacteria have prismatic crystals measuring 90×120 nm, aligned in a chain of average length 10 crystals. The bacterial crystals saturate at ~ 100 mT ($1 \text{ mT} = 10 \text{ Oe}$), with an intersection at ~ 50 mT. The coercivity spectrum of the Santa Barbara Basin material also saturates at ~ 100 mT and intersects at ~ 50 mT. Grain-grain interaction and the presence of other magnetic minerals (that is, coarse-grained magnetite and haematite) in the sediment may account for the differences between the two spectra¹³. A few particles of multi-domain magnetite ($> 1 \mu\text{m}$) were seen in sediment extracts. Figure 3 also shows (triangles) the coercivity spectrum of a marine sediment containing mostly coarse-grained magnetite and no bacterial magnetite. Both the saturation values and the intersection point are markedly different. This suggests that the coarse-grained component does not contribute significantly to the magnetic properties of the surface sediments.

The maximum values of sIRM obtained for both fixed and unfixed samples ranged between 2.5×10^{-4} and 1.25×10^{-3} e.m.u. g^{-1} and agree with previously published measurements for hemipelagic sediments^{1,2}. A magnetotactic bacterium with a chain of 20 cuboidal 50-nm crystals, such as those seen in the Santa Barbara bacteria, has a calculated saturation moment of 10^{-12} e.m.u. g^{-1} (refs 8, 10). If all the remanent magnetization is attributed to the bacteria, a population density of between 10^7 and 10^8 magnetotactic bacteria per gramme of sediment is predicted. Such a population was not seen in the material we examined; however, direct field counts at the time of collection could not be made. Populations of magnetotactic bacteria from other environments have been observed to decline dramatically within hours or days after collection¹². The discrepancy in the magnetic remanence and the actual number of live bacteria observed in the Santa Barbara sediments may be explained by episodic increases in population, the persistence of ultra-fine-grained magnetite in the surface sediments, the presence of other magnetic minerals (including some coarse-grained magnetite), and the death of much of the population during sample collection and storage. The bacteria contribute significantly to the natural magnetic remanence because each

of the crystals is single-domain and is usually deposited as part of a chain. The discovery of an intact magnetosome and chains of magnetite in the sediment extracts suggests that these crystals remain aligned even outside the bacteria. Unlike bacterial magnetites, coarse-grained magnetites have very little effect on the natural magnetic remanence.

This study has concentrated on the surface sediments of the Santa Barbara Basin, where the conditions of growth for the magnetotactic organisms may be optimal. A microbial mat community, dominated by species of *Beggiatoa*, a sulphur-oxidizing chemolithotroph, thrives on the surface (M. Kastner, personal communication). *Beggiatoa*, along with most magnetotactic bacteria, are microaerophiles, needing trace quantities of oxygen for their metabolism^{14,15}. The abrupt disappearance (within the top metre) of the ultra-fine-grained component in sediments from the California continental borderland²⁻⁴ and the Gulf of California^{1,3} can be explained by the disappearance of the magnetotactic bacteria and the dissolution of the bacterial magnetites. In these sediments iron oxide reduction is coupled with the diagenesis of organic material^{1,3}. The profiles of sulphur, sulphate and oxygen concentration support this explanation^{1,3,16}.

The results of this study and those we have conducted in two other marine environments (a lagoonal environment in Baja California, Mexico, and a carbonate environment in the Florida Keys, USA)¹² suggest that magnetotactic bacteria are the source of the primary remanence carrier (ultra-fine-grained, single-domain magnetite) in many marine sediments. The eventual fate of this biogenic magnetite depends on a number of post-depositional factors. In environments where there is a strongly reducing zone with abundant sulphide, only greigite pseudomorphs of magnetite have been found¹⁷. Maghemite pseudomorphs of bacterial magnetite have also been seen in deep-sea sediments¹⁸. The loss of the ultra-fine-grained bacterial magnetite component with depth in open ocean basins may limit the extent of the biogenic magnetite contribution to rock magnetism to marine sediments with low organic content such as calcareous oozes and limestones.

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Note added in proof: Bacterial single-domain magnetite in deep ocean sediments as old as 50 Myr has recently been reported by Petersen *et al.*¹⁹ and discussed by Frankel²⁰.

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Carbon isotope evidence for a magmatic origin for Archaean gold-quartz vein ore deposits

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Archaean gold-quartz vein/shear zone systems constitute one of the most important types of gold ore deposit; however, their origin is controversial. Here we discuss newly obtained $\delta^{13}\text{C}(\text{PDB})$ values for carbonate associated with the second largest such system in the world (Hollinger-McIntyre, Superior Province, Canada), together with values for fourteen other Au-mineralized locations in the Timmins area¹ and for the largest such system in the world (Golden Mile, Western Australia²). All of the data are statistically identical to the $\delta^{13}\text{C}$ values, reported here, of proven magmatic carbonate recently found with Au- and W-enriched MoS_2 mineralization in the Mink Lake sodic granodiorite stock, north-west Ontario. The $\delta^{13}\text{C}$ values appear to exclude greenschist-amphibolite facies metamorphic processes, a widely accepted genetic alternative³⁻⁶, and granulite facies processes^{7,8}. We conclude that currently available carbon isotope data suggest that the $\text{H}_2\text{O}-\text{CO}_2$ fluid which deposited this type of Archaean Au mineralization was magmatically derived.

The Hollinger (Timmins)-McIntyre (Schumacher)-Coniaraum-Vipond-Moneta-Crown vein system located in the Abitibi greenstone belt, ~550 km north-north-west of Toronto, is ~5 km long, a maximum of ~2 km wide, and has been mined to a depth of 2.4 km (7,875-ft level, McIntyre mine). End-1984 past-production plus reserves total 100.9 million tonnes of ore, at 9.9 g tonne⁻¹ recovered/recoverable Au and ~2 g tonne⁻¹ recovered/recoverable Ag^{9,10}. Of the 994.7 tonnes of contained Au, 60.2% occurred in the Hollinger deposit, which has been in production with some interruptions since 1910. Between 1940 and 1953 it was the principal source of scheelite (CaWO_4) of the gold mines in the Archaean of Canada (47,878.1 tonnes ore at 0.78% WO_3)¹¹⁻¹³. Thus the Hollinger system is an excellent example of the Archaean gold-quartz-(Fe-carbonate-pyrite $\pm$ tourmaline $\pm$ tellurides $\pm$ W (scheelite) $\pm$ Mo) type of vein deposit.

The principal type of mineralization in Hollinger is related to quartz-dominated veins with prominent pyrite-Fe-carbonate-muscovite alteration envelopes. The veins are geometrically unusual in that they consist of steeply dipping composite zones containing tabular sections, multiple curves from which extend

flats, curved branches, feathers with bifurcations, splits and spurs, and more complicated geometries, all of which largely cross-cut the local fabric. The bulk of the gold (~95%) occurs as small (~5-60 μm) particles within pyrite grains in the alteration selvages. Carbonate (20-60 mol % $\text{CaFe}(\text{CO}_3)_2$) is very closely related to gold mineralization, occurring with albite as internally projecting fringes on the margins of veins and, particularly, metasomatically dispersed in the mineralized wall-rock. The composition of the carbonate is zoned from Fe-rich in the core of the deposit to Fe-poor around the periphery (Fig. 1). The zone of mineralization and carbonate alteration narrows downwards to the root of the system (Fig. 1). Hollinger, therefore, exemplifies the remarkably ubiquitous association between carbonate and Archaean lode gold mineralization^{3-5,14-16}. The remainder of the vein-fill at Hollinger consists of minor sulphides (pyrite, sphalerite, chalcopyrite, pyrrhotite, arsenopyrite and galena), beige scheelite, and minor muscovite, chlorite, tourmaline, tellurides and native bismuth.

Carbon dioxide is known to be a major component of Archaean Au-quartz vein fluids⁵; for this reason, the carbon isotopic composition of the carbonate in the Hollinger deposit has been determined as a source tracer. Analysis of 14 vertically and laterally representative samples (Table 1, Fig. 2) yielded $\delta^{13}\text{C}$ values ranging from -4.8 to -0.1‰, with an arithmetic mean of $-3.0 \pm 1.5\%$ (1σ). Data for vein and wall-rock alteration carbonate are statistically identical (Kolmogorov-Smirnov test¹⁷) at -2.8 ± 1.8 and $-3.3 \pm 1.3\%$, an observation consistent with the two being cogenetic. The histogram in Fig. 2 shows that the Hollinger data are characterized by a marked positive skewness and, consequently, a mode of -4.3‰ which is lower than the arithmetic mean. Thirty previous carbonate $\delta^{13}\text{C}$ determinations for the Hollinger system¹ define a higher arithmetic mean of $-0.6 \pm 0.5\%$ and a range of -1.5 to +0.3‰, which overlaps the top end of the range defined in this study. The samples were obtained from only two pits at the surface (55 and 68) and have been described as coming from "stratabound pyritic 'flow-ore', and stratiform pyritic graphitic units"¹⁸, which are not the major ore types in the Hollinger deposit. This study contains data for two samples from pit 55 (Table 1). They are relatively heavy (-1.1 and -1.0‰), and are thus consistent with the determinations in the previous study¹. It appears, therefore, that the previous data were obtained from samples which were unrepresentative of the system as a whole.

Values (275 data points) for a further 14 Au-mineralized locations in the Timmins area¹ (for example, Aunor, Buffalo Ankerite and Delnite mines, but data for Hallnor not included because of the presence of graphitic inter-flow sediments which could modify the $\delta^{13}\text{C}$ values¹) define a range and arithmetic mean which are very similar to the new Hollinger data: -6.1 to +0.8‰ with an outlier at +2.2‰, compared with -4.7 to -0.1‰ (Hollinger), and $-3.1 \pm 1.3\%$, compared with $-3.0 \pm 1.5\%$ (Hollinger). As the histogram in Fig. 2 shows, the data for the 14 other Au-mineralized localities in the Timmins area are also asymmetrically distributed, with a distinct positive skewness. A χ^2 test confirms that the data are not normally distributed ($\chi^2_{\text{calculated}} = 64.3$; $\chi^2_{0.05,9} = 16.9$) and that, therefore, parametric statistical significance tests are inappropriate. Hence, a two-tailed Kolmogorov-Smirnov test^{19,20} was used to verify the hypothesis that the new Hollinger samples and the sample for the 14 Timmins-area Au locations are the same (see ref. 17 for details). A Kolmogorov-Smirnov test was chosen because it is sensitive to any difference in the distributions from which two samples have been drawn.

The $\delta^{13}\text{C}$ values for the pooled data (new Hollinger plus 14 selected Timmins-area Au locations: $\bar{x} \pm 1\sigma = -3.1 \pm 1.3\%$; $n = 289$) may be compared with $\delta^{13}\text{C}$ values for magmatically derived carbonate found recently²¹ in association with Au- and W-enriched MoS_2 mineralization in the Mink Lake granodiorite intrusion. This 1.5×3.25 km stock located ~110 km north-east of Red Lake is an unmetamorphosed, completely post-tectonic

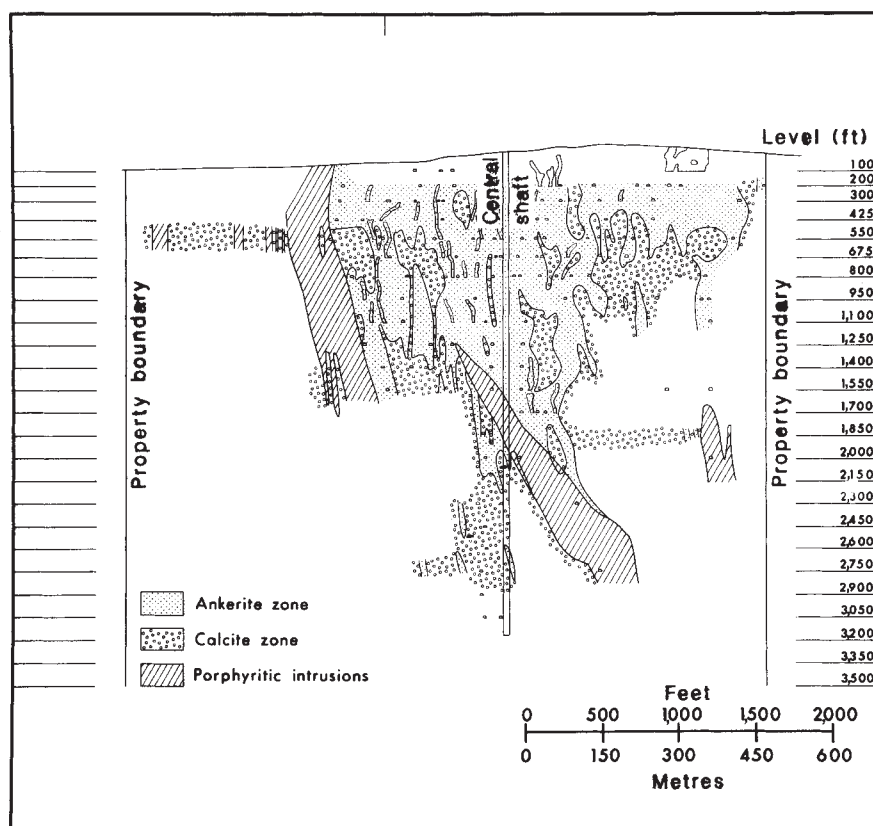


Fig. 1 Vertical cross-section of the Hollinger Au-vein deposit (through 9 cross-cut), showing a zonation from ankeritic carbonate in the core to calcite around the edges, and the narrowing of the mineralized zone downwards to the root of the system.

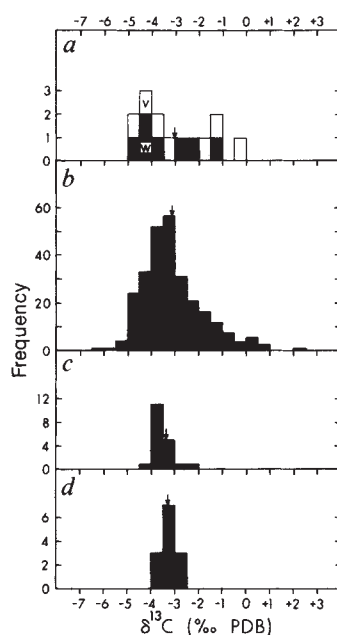


Fig. 2 Histograms of the carbonate $\delta^{13}\text{C}$ data. The histogram and statistics for the 14 Au-mineralized locations in the Timmins area¹ do not include the data for Hollinger or Hallnor in ref. 1 because of the presence of graphitic inter-flow sediments in the sampled areas. As suggested in ref. 1, such material could modify $\delta^{13}\text{C}$ values. Data for the No. 4 lode, Golden Mile, Kalgoorlie, Western Australia are from ref. 2. v, Vein material; w, altered wall-rock. Arrows denote arithmetic means. a, Au vein mineralization, Hollinger mine, Timmins, northern Ontario; $\bar{x} = -3.0 \pm 1.5$ (1σ); $n = 14$. b, Au vein mineralization, Timmins area (14 locations, excluding Hollinger); $\bar{x} = -3.1 \pm 1.3$; $n = 275$. c, Au vein mineralization, No. 4 lode, Golden Mile, Kalgoorlie, Western Australia; $\bar{x} = -3.4 \pm 0.4$; $n = 19$. d, MoS_2 mineralization, Mink Lake granodiorite stock, northwestern Ontario; $\bar{x} = -3.3 \pm 0.4$; $n = 13$.

intrusion in the Archaean Uchi sub-province²¹. It is a sodic granodiorite in composition ($\text{SiO}_2 = 64\text{--}71\%$; $\text{Na}_2\text{O}/\text{K}_2\text{O} = 1.6\text{--}3.2$), and contains a $350 \times 1,000$ m zone of MoS_2 mineralization and associated carbonate metasomatism completely enclosed within its southern end. Carbonate alteration zones occur as symmetrically developed selvages adjacent to aplites ($\text{SiO}_2 = 75\text{--}76\%$) and quartz-carbonate $\pm$ K-feldspar-chlorite-pyrite veins, both of which contain MoS_2 , and as larger, but localized, sub-horizontal, tabular zones up to 3 m in width also containing MoS_2 , but lacking internal veins. The MoS_2 mineralization and carbonate alteration are considered to be magmatically derived for a number of reasons (discussed in detail in ref. 22): (1) they are directly related to an observable silicate melt-hydrothermal fluid(s) transition, as MoS_2 and carbonate occur in, and carbonate occurs adjacent to, aplites of granitic composition which represent late melt, as well as quartz-dominated hydrothermal veins; (2) hydrothermal fluids trapped in $5\text{--}20\text{-}\mu\text{m}$ fluid inclusions are CO_2 -rich and show low aqueous-phase salinities of 5.6 ± 1.2 equiv. wt % NaCl ($n = 48$)²³, both attributes being characteristic of fluids shown to be magmatic in other cases (for example, Boss Mountain Mo deposit, British Columbia²⁴, Tanco Ta (-Sn) granitic pegmatite, south-east Manitoba²⁵); (3) the fluid δD - $\delta^{18}\text{O}$ box estimated from the bulk of the δD and $\delta^{18}\text{O}$ data shows a $\sim 60\%$ overlap with the general magmatic water field but a significantly smaller overlap ($\sim 18\%$) with the general metamorphic water field, and no relationships to sea water or meteoric water values; (4) the MoS_2 mineralization is completely contained within the stock with no obvious external connections; (5) the intrusion is in any case post-tectonic and post-metamorphic; (6) the mineralization developed marginal to the principal internal zone of fractional crystallization, as shown by contoured Rb, TiO_2 , Zr and Li data ($n = 47$); and (7) the aplites, the compositions of which are geochemically enriched in Au, Mo and W, thus confirming a link with the subsequent Au- and W-enriched MoS_2 mineralization.

Table 1 $\delta^{13}\text{C}$ values for Hollinger and Mink Lake carbonate

Sample no.	Sample description	$\delta^{13}\text{C}$ (‰ PDB)
Au-quartz mineralization, Hollinger mine		
33P-S342-v	Vein in volcanic fragmental unit, NE end of pit 33, N part of deposit.	-3.8
33P-S342-w	Wall-rock; as above.	-3.7, -3.6
55P-S330-v	Vein in mafic flow (no. 99), SW end of pit 55, S part of deposit.	-1.0
55P-2-w	Wall-rock; as above.	-0.9, -1.2
4-S124-v	Vein in strained Pearl Lake porphyritic intrusion, 425-ft level, NE part of deposit.	-1.8, -2.0
4-S130-w	Wall-rock in mafic volcanic (M1), 425-ft level, SW part of deposit.	-4.1, -4.0
8-S310-v	Vein in mafic volcanic, 800-ft level, SW part of deposit.	-0.1, -0.1
8-S310-w	Wall-rock; as above.	-2.2, -2.2
12-S166-v	Vein in strained Pearl Lake porphyritic intrusion, 1,250-ft level, NE part of deposit.	-3.3, -3.4
15-22-w	Wall-rock in strained Millerton porphyritic intrusion, 1,500-ft level, SW part of deposit.	-4.3, -4.3
18-83-01-v	Vein in strained Millerton porphyritic intrusion, 1,850-ft level, SW part of deposit.	-5.0, -4.6
18-16-w	Wall-rock; as above.	-4.6
27-S158-v	Vein in strained Millerton porphyritic intrusion, 2,750-ft level, central part of deposit.	-4.5, -4.2
27-S155b-w	Wall-rock in strained Millerton porphyritic intrusion, 2,750-ft level, SW part of deposit.	-2.8, -2.7
$\bar{x} \pm \sigma$		-3.0 $\pm$ 1.5
MoS₂ mineralization, Mink Lake stock		
P-75	Fe-calcite in quartz-chl clot in felsic pegmatite vein cut by early aplite.	-3.5, -3.4
S-32	Carbonate alteration selvage on thin MoS ₂ -bearing aplite.	-3.4, -3.2
		-3.5, -3.4
S-21	Carbonate alteration; no associated quartz vein; cc-chl-MoS ₂ -py.	-2.7
S-109	Central MoS ₂ -rich band of 120-cm-wide carbonate alteration zone.	-3.2
S-25/L128	White carbonate from within chlorite haloes; MoS ₂ -bearing carbonate alteration.	-2.8
L-113b	Light buff carbonate alteration; chl haloes similar to S-25; MoS ₂ -py, no associated quartz vein.	-2.8
C-29	Unmineralized carbonate alteration.	-3.8, -3.7
S-19	Chl-orthoclase-cc-MoS ₂ quartz vein. Py cubes up to 5 mm.	-3.1, -3.1
S-97	Carbonate alteration selvage on quartz vein; MoS ₂ -chl-py.	-3.9
S-9	Mineralized carbonate alteration.	-3.5, -3.4
S-4/L104	Carbonate alteration selvage on quartz vein with cc, chl, orthoclase, py and MoS ₂ .	-3.5, -3.4
S-2	Carbonate alteration on MoS ₂ quartz vein.	-3.5, -3.4
S-81b	Late muscovite-chl alteration	-3.9, -3.7
$\bar{x} \pm \sigma$		-3.3 $\pm$ 0.4

$\delta^{13}\text{C}$ analyses were carried out at the Isotope Laboratory, Department of Earth Sciences, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada by standard techniques. The estimated routine uncertainty is $\pm 0.2\%$ (1σ). Precision, as defined by the mean average deviation for the 20 duplicated analyses in this study, is $\pm 0.1\%$ ($\pm 3.1\%$), where mean average deviation is defined as $(\sum_{i=1}^n \Delta_i / 2 \cdot \bar{x}_i) / n$ (n = number of duplicates; Δ_i = difference, regardless of sign, for the i th pair; $\bar{x}_i$ = arithmetic mean of the i th pair)³⁶.

The samples from Hollinger are equally divided between vein and metasomatic wall-rock carbonate, and were selected to be vertically and laterally representative of the deposit. Hence, samples were obtained from pits 33 and 55 at the surface, and the 425-, 800-, 1,250-, 1,550-, 1,850- and 2,750-ft levels underground. Sampling below the 2,750-ft level was not possible because the lower levels, which extend down to 5,450 ft, were no longer accessible. Lateral sample positions on the different levels were variable (see above). Host lithologies are either strained mafic flows or strained porphyritic intrusions (see above). Samples from the high-grade core zone of the Hollinger deposit were very difficult to collect because of inaccessible collapsed workings. One sample (27-S158-v) was obtained. Most samples come from the southwestern (SW), northeastern (NE), southern (S) and northern (N) parts of the deposit. cc, Calcite; chl, chlorite; py, pyrite.

Thirteen $\delta^{13}\text{C}$ determinations for Mink Lake carbonate from various associations (Table 1) define a very narrow range from -3.9 to -2.7‰, which is completely enclosed within the pooled Timmins-area Au/Mink Lake MoS₂ carbonate $\delta^{13}\text{C}$ values in 16 locations constitute very strong evidence against natural mixing processes which give irregular ranges. Granulite facies metamorphic processes appear to be excluded by fluid inclusion CO₂ $\delta^{13}\text{C}$ values, ranging from -21.9 to -4.5‰ (n = 10; ref. 31), and, particularly, by the extremely CO₂-rich fluid compositions which are characteristic of granulites, including those in the Superior Province^{22,32,33}. Finally, the carbon species in the fluids are dominated by CO₂, so that intercomparison of carbonate $\delta^{13}\text{C}$ values is valid (for example, Hollinger CO₂ melting point (m.p.) = $-56.7 \pm 0.7^\circ\text{C}$ (1σ), n = 100 (ref. 34); Mink Lake CO₂ m.p. = $-56.4 \pm 0.8^\circ\text{C}$, n = 57 (refs 21, 22); pure CO₂ m.p. = -56.6°C). The depositional temperatures are also very similar, so that CO₂-carbonate $\delta^{13}\text{C}$ fractionations are comparable (Hollinger, $271 \pm 47^\circ\text{C}$ (ref. 34); Mink Lake, $298 \pm 37^\circ\text{C}$ (refs 21, 22)).

Thus, the weight of the currently available carbon isotope evidence indicates that Archaean gold-quartz vein mineralization is magmatically derived, supporting the suggestion of Wood *et al.*³⁴ that components of domal tonalite gneiss-granodiorite-quartz monzonite-type material which intrudes the lower parts of Archaean greenstone belts could constitute an indirect, or direct, source region.

mainly between -35 and -20‰ (ref. 30). Mixtures are possible; however, the very narrow ranges and, above all, the recurrence of the Timmins-area Au/Mink Lake MoS₂ carbonate $\delta^{13}\text{C}$ values in 16 locations constitute very strong evidence against natural mixing processes which give irregular ranges. Granulite facies metamorphic processes appear to be excluded by fluid inclusion CO₂ $\delta^{13}\text{C}$ values, ranging from -21.9 to -4.5‰ (n = 10; ref. 31), and, particularly, by the extremely CO₂-rich fluid compositions which are characteristic of granulites, including those in the Superior Province^{22,32,33}. Finally, the carbon species in the fluids are dominated by CO₂, so that intercomparison of carbonate $\delta^{13}\text{C}$ values is valid (for example, Hollinger CO₂ melting point (m.p.) = $-56.7 \pm 0.7^\circ\text{C}$ (1σ), n = 100 (ref. 34); Mink Lake CO₂ m.p. = $-56.4 \pm 0.8^\circ\text{C}$, n = 57 (refs 21, 22); pure CO₂ m.p. = -56.6°C). The depositional temperatures are also very similar, so that CO₂-carbonate $\delta^{13}\text{C}$ fractionations are comparable (Hollinger, $271 \pm 47^\circ\text{C}$ (ref. 34); Mink Lake, $298 \pm 37^\circ\text{C}$ (refs 21, 22)).

Thus, the weight of the currently available carbon isotope evidence indicates that Archaean gold-quartz vein mineralization is magmatically derived, supporting the suggestion of Wood *et al.*³⁴ that components of domal tonalite gneiss-granodiorite-quartz monzonite-type material which intrudes the lower parts of Archaean greenstone belts could constitute an indirect, or direct, source region.

This result may have broader applicability. The 1.2×3 km set of ~ 300 shear zone lodes which constitutes the Golden Mile in the Norseman-Wiluna greenstone belt in the Archaean Yilgarn block of Western Australia is the largest known Archaean gold-quartz system in the world, having produced $\sim 1,200$ tonnes of Au³⁵ (compare with the Hollinger system total of 994.7 tonnes Au). The carbon isotopic composition of the associated Fe-carbonate, $-3.4 \pm 0.4\%$ (Fig. 2), as defined by 19 determinations from two traverses of the No. 4 lode (DDH 5027 and DDH 5022)², is closely similar to that of the pooled Timmins-area Au locations ($-3.1 \pm 1.3\%$), and remarkably similar to the Mink Lake stock MoS₂-associated carbonate ($-3.3 \pm 0.4\%$). Two-tailed Kolmogorov-Smirnov tests confirm that all of the data are drawn from the same population at the 5% significance level¹⁷. Hence, the No. 4 lode, Golden Mile $\delta^{13}\text{C}$ data are also consistent with a magmatic derivation.

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Significance of a $\delta^{13}\text{C}$ anomaly near the Devonian/Carboniferous boundary at the Muhua section, South China

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The Muhua section of Guizhou Province, South China is one of three candidates for the Devonian/Carboniferous international boundary stratotype¹. Although palaeontological and stratigraphical data have been published^{2,3}, no stable isotope study has previously been made. Detailed $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analyses from the Muhua section reveal a significant short-term $\delta^{13}\text{C}$ negative anomaly of 8-9‰ at the base of the *Siphonodella crenulata* conodont zone. This is the first $\delta^{13}\text{C}$ negative anomaly found near the Devonian/Carboniferous boundary, and is similar to $\delta^{13}\text{C}$ anomalies reported from the Permian/Triassic, Cretaceous/Tertiary, and Precambrian/Cambrian boundaries^{4,5}. We suggest, therefore, that the $\delta^{13}\text{C}$ anomaly at Muhua section provides evidence for major rapid changes in ocean-atmospheric chemistry, and may reflect an important event in geological history.

Muhua town, Changshun County is located ~ 100 km south of Guiyang, Guizhou Province, South China (Fig. 1). The Muhua section has been investigated by both indigenous and foreign palaeontologists and stratigraphers, including W. Zeigler, C. A. Sandberg, E. Papproth and M. Streel. We have studied Muhua section II. Details of the stratigraphy and fossil zonation of the Muhua section are given in ref. 2 and summarized in Fig. 2.

The section sampled was laid down in an offshore to deep-water facies consisting of grey or black, bedded or nodular limestone and argillaceous limestone, rich in conodonts and with a few ammonoids. During fieldwork in 1984 and 1985, more than 100 samples were collected, in ascending order at intervals of 10-50 cm over a thickness of 16 m.

Fresh samples without late calcite veins were analysed for carbon and oxygen isotopes. All analyses reported in this study were made on whole-rock samples, all of which are carbonates. The analyses were performed using modern techniques^{6,7}. The samples were treated under vacuum with 100% phosphoric acid at 75 °C. The evolved CO₂ was separated, purified and analysed



Fig. 1 Sketch map of the location of Muhua section II (**).

for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in a triple collecting mass spectrometer MAT 251. Reference samples (working standard) used in the analytical procedure were CY1 (carbonate), NBS18 and NBS19. The analytical precision of the C and O isotope data (2σ) was better than 0.1‰ (ref. 7).

Figure 2 shows the changes in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values in the Muhua section. The $\delta^{13}\text{C}$ values in the Daihua and Wangyou Formations are 0–2.3‰ positive, and suddenly decrease to negative values in the lower part of the Muhua Formation. A minimum carbon isotope anomaly with a value of –8.6‰ is found near the base of the Muhua Formation (Bed 65). Thus, the total range of change in $\delta^{13}\text{C}$ is ~8–9‰.

The change in $\delta^{18}\text{O}$ is positively correlated with that of $\delta^{13}\text{C}$ below Bed 57, but above Bed 64 $\delta^{18}\text{O}$ values suddenly increase and become negatively correlated with corresponding values of $\delta^{13}\text{C}$ (Fig. 2). The sudden change in the relationship of $\delta^{13}\text{C}$ to $\delta^{18}\text{O}$ after deposition of Bed 64 indicates a severe environmental change during the earliest stage of sedimentation of the Muhua Formation.

The change in $\delta^{13}\text{C}$ in the Muhua section is similar to patterns of variation across other important boundaries in the Phanerozoic. Stable isotope data^{8,9} indicate a 6–7‰ negative $\delta^{13}\text{C}$ anomaly across the Permian/Triassic boundary in the Shangsi section, Sichuan Province, and a 5–6‰ negative shift above the Precambrian/Cambrian boundary has been found^{4,5}. Perch-Nielsen *et al.*¹⁰ suggested that there was a 1–2‰ negative $\delta^{13}\text{C}$ anomaly above the Cretaceous/Tertiary boundary in a time range of 10^5 yr. Carbon isotope analyses of the Frasnian/Famennian boundary show a drop in overall $\delta^{13}\text{C}$ of ~1.5‰ (ref. 11).

The uptake of light carbon by photosynthetic organisms leads to a decrease in ^{12}C in carbonates, and thus a mass extinction of biomass would decrease the $\delta^{13}\text{C}$ value. Following a geologically brief period, the surviving community would bloom and the $\delta^{13}\text{C}$ value would gradually become positive. Hence, a large $\delta^{13}\text{C}$ negative anomaly immediately above a geological boundary suggests a suspension of biotic productivity in the surface water, and a significant change in the biomass of the ancient ocean^{2,4}.

The $\delta^{13}\text{C}$ anomalies at the Cretaceous/Tertiary, Permian/Triassic and Precambrian/Cambrian boundaries coincide stratigraphically with mass extinctions and iridium anomalies, and it has been suggested that they are caused by rare events of short duration⁴. A similar pattern of $\delta^{13}\text{C}$ variation in the Muhua section further suggests that an important event occurred near the base of the Muhua Formation.

The sedimentation rate of the Devonian and Carboniferous carbonates in the Muhua section cannot be accurately determined. Playford *et al.*¹¹ estimate an average time span for an Upper Devonian conodont subzone of ~500,000 yr, and thus suggest an average rate of sedimentation of 1.8 mm kyr^{-1} . The conodont zones in the Wangyou and Muhua Formations are developed in sedimentary units of comparable thickness to those of the Canning Basin zones. We therefore suggest that the 90-cm interval containing the major negative change in $\delta^{13}\text{C}$ in the Muhua section may represent ~500,000 yr.

Although the Working Group on the Devonian/Carboniferous boundary has reached a consensus on the biostratigraphical criteria to be used in the identification and correlation of the boundary level, other methods have also been recommen-

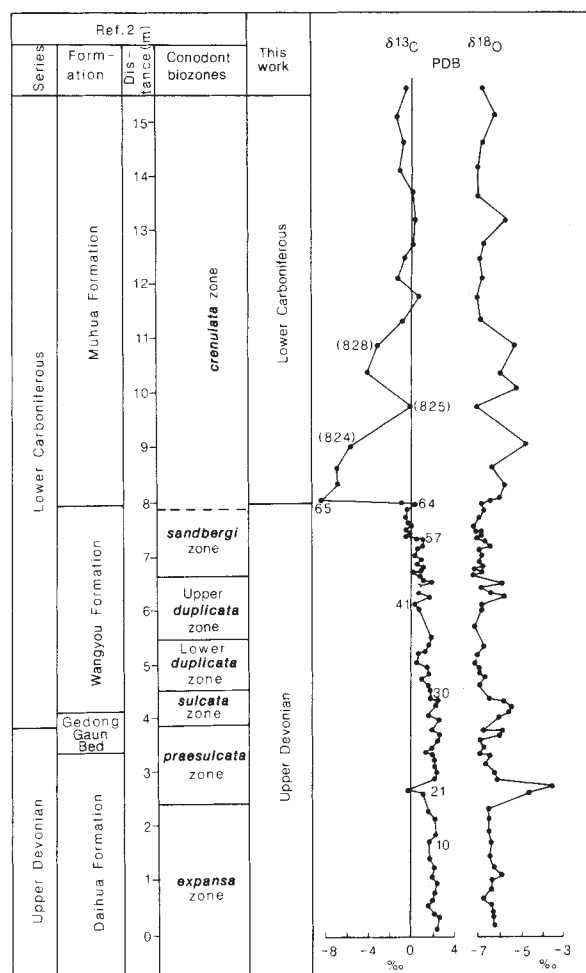


Fig. 2 Stable isotope descriptions of Muhua section II. Numbers on curves are the numbers of the beds from ref. 2. Numbers in parentheses are our sample numbers.

ded¹, including geochemistry and petrology. A major requirement for defining a natural boundary between systems is the possibility of accurately correlating that horizon with other areas and continents.

The negative $\delta^{13}\text{C}$ anomaly provides evidence of a sudden decrease in fertility of organisms in the ancient ocean, and may satisfy such a requirement. We suggest, therefore, that the results of the present study define a natural boundary at the base of the negative $\delta^{13}\text{C}$ anomaly (Bed 65). According to the researches of conodont zonation, the above-mentioned position is probably correlated to the lowest part of *S. crenulata* Zone. Unfortunately, *S. crenulata* has not been found in the Muhua section¹.

More Devonian/Carboniferous sections will be sampled and analysed to verify the anomaly at this horizon.

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Organically preserved microbial endoliths from the late Proterozoic of East Greenland

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Diverse microorganisms ranging from cyanobacteria to eukaryotic algae and fungi live endolithically within ooids, hardgrounds and invertebrate shells on the present-day sea floor. These organisms are involved in the mechanical destruction of carbonates, and are useful ecological indicators of water depth and pollution¹⁻³. The Phanerozoic history of microbial endoliths has been elucidated through the study of microborings (the trace fossils of endolithic microorganisms) and rare cellularly preserved individuals^{1,3,4}, but nothing was known of the possible Precambrian evolution of comparable microorganisms until Campbell⁵ documented the occurrence of microborings in late Proterozoic ooids from central East Greenland. We now report the discovery of large populations of organically preserved endolithic microorganisms in silicified pisolites from the 700–800-Myr-old Limestone–Dolomite Series of East Greenland. This fossil assemblage is significant for three reasons: (1) It confirms the prediction⁵ that oolites, pisolites and hardgrounds—the substrates for pre-Phanerozoic endoliths—provide a hitherto poorly explored but rewarding set of environments into which the search for early microfossils must be broadened; (2) the assemblage is diverse, containing about 12 taxa of morphologically distinct and previously unknown endolithic cyanobacteria, plus associated epilithic and interstitial populations; and (3) at least six of the fossil populations are indistinguishable in morphology, pattern of development, reproductive biology and inferred ecology from distinctive cyanobacterial species that bore ooids today in the Bahama Banks.

The Eleonore Bay Group comprises some 7,000 m of unmetamorphosed sedimentary rocks that lie beneath latest Proterozoic glaciogenic beds in the coastal fjordlands of central East Greenland⁶⁻⁸. Carbonates occur principally in the upper part of this sequence, particularly in the 1,200-m-thick Limestone–Dolomite 'Series'. The Limestone–Dolomite succession documents a prolonged period of carbonate deposition along an extensive peritidal platform not unlike that of the present-day Bahama Banks. Flat-laminated, hemispherical and columnar stromatolites abound in this sequence, and these are intimately associated with oolites, pisolites, catagraphs, intraformational conglomerates and rippled to flat-laminated carbonate mudstones⁹. On biostratigraphic grounds, the Limestone–Dolomite sequence is considered to be predominantly late Riphean (perhaps 700–800 Myr)¹⁰, although the uppermost beds of the unit, above the horizons of interest here, may be Vendian.

The fossils in question occur in silicified pisolites from Bed 18 of the Limestone–Dolomite Series exposed on Ella Ø (72°53' N, 25°8' W) and 87 km to the north in exposures along the east flank of Grejsdalen (73°33' N, 25°5' W). Individual pisids within the pisolites average 6–9 mm in diameter, but grains up to 14 mm have been observed. In both localities, the pisolites are cross-bedded, rippled and well-sorted, often grading into conventionally sized oolites. Although the pisids have been affected to various extents by silicification, neomorphism, dolomitization and calcitization, petrographic fabric analysis and strontium content determinations suggest that they consisted originally of concentric laminae of tangentially oriented aragonite crystals⁹. In all sedimentological, petrographic and

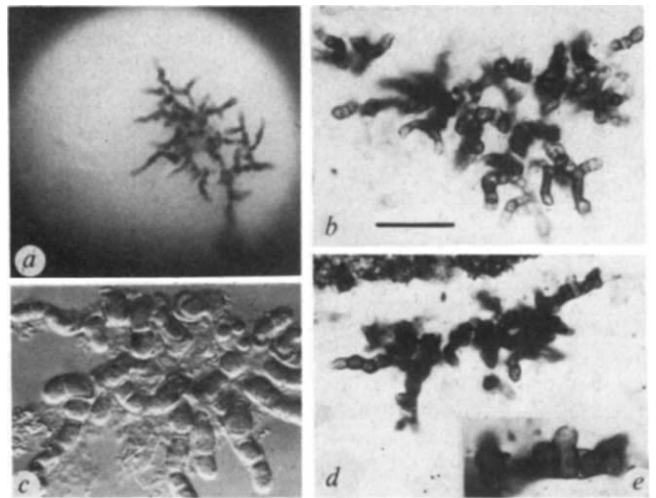


Fig. 1 a, Modern endolithic cyanobacterium *Hyella gigas* in ooid from the Bahama Banks ($\times 90$; scale bar in b = 115 μm). b, d, Fossil endolithic microorganisms comparable in morphology, development, reproduction and inferred ecology to modern *H. gigas* ($\times 128$; scale bar in b = 80 μm). c, Modern *H. gigas* freed from ooid to show details of morphology ($\times 255$; scale bar in b = 40 μm). e, Detail of d showing branch initiation ($\times 500$; scale bar in b = 40 μm).

micropalaeontological aspects, the pisids are essentially large ooids and not diagenetic structures formed in the vadose zone⁹. Chert occurs as irregular nodules that loosely parallel bedding; field and petrographic observations indicate that the silica is an early diagenetic replacement of primary carbonate sediments⁹. All organically preserved endoliths occur within the outer rim of individual pisids, suggesting that preservation was made possible by the rapid burial of pisids following a period of extensive endolithic colonization.

Among the more common constituents of the endolith assemblage is a population of relatively large, branching filamentous microfossils that radiate inward from their points of entry into pisolites (Fig. 1b, d). Like most other fossils in the Limestone–Dolomite assemblage, these specimens are preserved largely as extracellular envelopes that only rarely contain highly degraded cell contents; however, envelope morphologies preserve a record of divisional patterns and approximate original cell size. Filaments are predominantly uniserial, 8.5–101 μm long (mean 42.5 μm ; s.d. 23.8 μm ; $n = 37$) by 7.5–21 μm wide (mean 12 μm ; s.d. 2.5 μm), and consist of the extracellular envelopes of coccolidal cells 8.5–21 μm long (mean 13 μm ; s.d. 3.5 μm ; $n = 125$) by 6.5–21 μm wide (mean 11 μm ; s.d. 2 μm). Terminal cells may be slightly longer than intercalary cells. Because the cells of endolithic cyanobacteria are constrained by their extracellular envelopes and essentially do not move after division, developmental pattern can be reconstructed from individual specimens¹¹ and corroborated by observations of younger specimens in the same assemblage. Filaments evidently grew by transverse cell division and branching originated by cell slippage within filaments and by longitudinal divisions of intercalary cells (Fig. 1e). Reproduction occurred by the formation of baeocytes (reproductive cells produced by repeated binary divisions without intervening cell growth) from cells near the pisid surface.

In cell and envelope size and shape, branching morphology, developmental pattern and mode of reproduction, these fossils are indistinguishable from populations of the modern endolithic cyanobacterium *Hyella gigas* Lukas and Golubic¹² which occur today as endoliths in oolitic shoals of the Bahama Banks (Fig. 1a, c). Marine ooids have narrowly circumscribed conditions of formation, and except for the more vigorous agitation implied by their larger size, the East Greenland pisolites appear

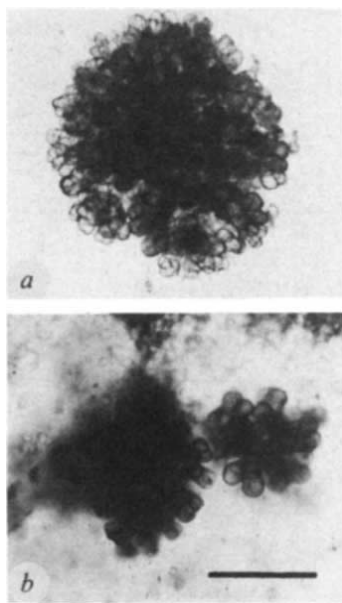


Fig. 2 *a*, Undescribed modern endolithic cyanobacterium freed from Bahamian ooid; view is essentially that seen from the interior of the substrate ($\times 140$; scale bar in *b* = 100 μm). *b*, Two specimens of fossil endolithic microorganisms comparable in morphology, development and inferred ecology to the living population illustrated in *a*; the fossil on the left is seen in longitudinal view, while the smaller individual on the right is viewed from the direction of the pisolith interior ($\times 350$; scale bar, 40 μm).

to have accumulated under sedimentary conditions much like those found today in the Bahamas. Thus, the morphological and reproductive similarity between *H. gigas* and its fossil counterpart is paralleled by a close environmental congruence between the modern and Proterozoic sediments in which the two taxa have been found.

Other fossil populations in the Limestone-Dolomite assemblage also compare closely with extant microorganisms that occur in Bahamian ooids. These include fossils resembling the modern taxa *Plectonema terebrans*, *Cyanosaccus* spp., *Solentia foveolarum* and unnamed coccoidal endoliths described by Harris *et al.*¹³. Perhaps the most interesting population is one composed of densely branched microfossils like that shown in Fig. 2*b*. These fossils have a high cell density, giving the appearance of a hemispherical cell cluster from which many short filaments radiate into the pisolite. Filaments are uniseriate or biserial, only a few cells long, and often terminated by a pair of end cells. Branches tend to be club shaped, widening towards the pisolite interior. Filaments are 20–47 μm long (mean 33.5 μm , s.d. 8 μm ; $n=20$) and have a maximum width of 12.5–16 μm (mean 14.5 μm ; s.d. 1.5 μm). Cell dimensions are 8.5–21 by 5–19 μm (mean $12.3 \times 12 \mu\text{m}$; s.d. $3.4 \times 3.7 \mu\text{m}$; $n=62$). Baeocyte formation has not been demonstrated for this fossil. This taxon is a common constituent of the East Greenland endolith assemblage, but it has no precise counterpart among described species of modern endolithic cyanobacteria. Our comparative studies of Bahamian ooids have revealed the presence of a locally common and hitherto undescribed blue-green (Fig. 2*a*) that is remarkably similar in morphology and development to the fossil population in question. This demonstrates that palaeobiological knowledge of late Proterozoic microfossils has become sufficiently refined to be of use to systematic and ecological studies of modern coastal marine microorganisms.

Equally well-preserved remnants of at least five other intertidal and shallow subtidal communities occur in silicified carbonates and shales of the Limestone-Dolomite Series¹⁴. Although the organisms in these assemblages lived con-

temporaneously with those found in the pisolites, there is essentially no taxonomic overlap between the endolithic and other biotas. Endolithic microfossil assemblages clearly document communities that differed significantly from those of the microbial mat and phytoplankton biotas usually reported from Precambrian rocks; thus, they add significantly to our knowledge of early microbial diversity.

The Limestone-Dolomite fossil endolith assemblage provides new and compelling examples of the close resemblance between Proterozoic prokaryotes and their modern counterparts^{15–17}, in this case a comparison that begins with morphology and extends to reproductive, developmental and ecological similarity. Most important, the comparisons relate several co-occurring fossils from a single Proterozoic assemblage to taxa that occur today as part of a single community in a physically equivalent environment. Collectively, Limestone-Dolomite fossils demonstrate that endolithic cyanobacteria were abundant, diverse and apparently quite modern in shallow marine environments well before the radiation of either grazing or skeletonized metazoans.

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More than one event in the late Triassic mass extinction

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The recent hypothesis that mass extinctions are discrete phenomena that have occurred with great regularity during the history of life^{1,2} is testable in several ways. Two essential elements of the hypothesis are (1) that each extinction event represents a significant departure from normal, or 'background', rates of extinction, and (2) that such mass extinction events are spaced equally in time. The analyses of the cyclicity of mass extinctions so far have concentrated on the past 250 Myr, with the first event occurring at the Permian-Triassic boundary, 245 Myr ago^{1–5}. The second event followed 26–33 Myr later^{1–6}, in the late Triassic. Here I present a detailed analysis of the fossil record of marine and non-marine life during the late Triassic which suggests that there were at least two phases of mass extinction during that time, separated by 12–17 Myr.

The late Triassic has long been recognized as an important time of mass extinction^{7–11} (approximately equivalent in magni-

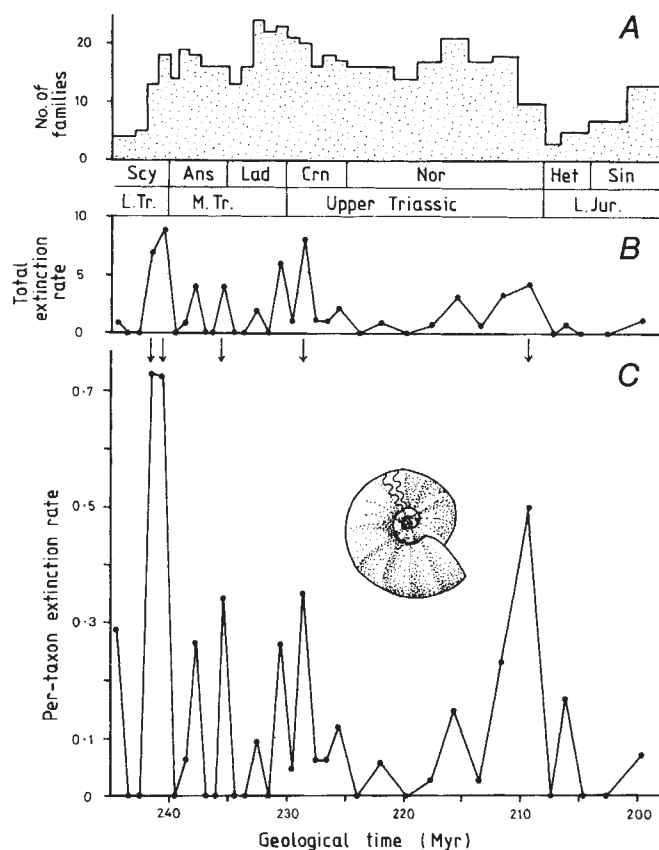


Fig. 1 Total diversity (A), total extinction rates (B) and per-taxon extinction rates (C) of families of Triassic ammonoids. The total extinction rates were calculated as the numbers of families dying out per Myr, and the per-taxon extinction rates were calculated according to the Van Valen metric²³, that is, the total extinction rate divided by the number of taxa at risk. (The latter was estimated as the number of taxa at the beginning of the interval, minus half the extinctions, plus half the originations in the interval.) The five highest per-taxon extinction rates (possible mass extinctions) are indicated by arrows. Data on ammonoid distributions taken from refs 12 and 22, and the timescale from ref. 21. The Triassic and earliest Jurassic subseries and stages are indicated. Diversity and extinction rate data were calculated by substages (Scythian, Hettangian, Sinemurian) and by zones (Anisian–Norian). The 'Rhaetian' stage is included in the late Norian¹². The fossil shown is *Ceratites*. Ans, Anisian; Crn, Carnian; Het, Hettangian; Lad, Ladinian; L. Jur., Lower Jurassic; L. Tr., Lower Triassic; M. Tr., Middle Triassic; Nor, Norian; Scy, Scythian; Sin, Sinemurian.

tude to the famous event at the end of the Cretaceous¹⁰, when the dinosaurs died out), and involved an overall reduction in the diversity of families in the sea of ~23% (ref. 10) with a similar reduction in diversity of families on the land. The main groups that were affected in the sea were the sponges (loss of 8 families), the gastropods (13 families lost), the bivalves (8), the cephalopods (58), the brachiopods (12) and various marine reptiles (13). The effects on the ceratid cephalopods, which were abundant and widespread in the Triassic, were dramatic (all 46 late Triassic families died out) and the ammonoids barely survived into the Jurassic¹². When genera are considered, the *Ceratitida* reached a peak of ~150 genera in the Carnian, falling to ~100 genera in the Norian, and to single figures in the latest Norian¹³. The extinction rate for bivalve families was not so marked, but the extinction rate for genera was 42%, and the species extinction rate, in Europe at least, was 92% (ref. 11). The last strophomenid brachiopods, conodonts, conulariids,

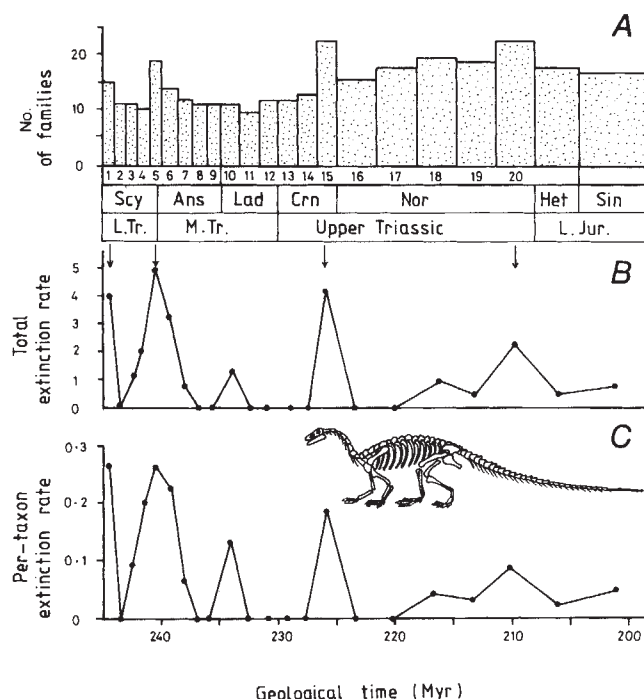


Fig. 2 Total diversity (A), total extinction rates (B) and per-taxon extinction rates (C) of families of Triassic and earliest Jurassic non-marine tetrapods. The four highest total extinction rates (possible mass extinctions) are indicated by arrows. The timescale is subdivided into informal 'substages', numbered 1–20 in the Triassic according to ref. 24. Data on tetrapod distributions are taken from refs 17, 18 and 24, with biostratigraphic zonations revised according to refs 25 and 26.

nothosaurs and placodonts also disappeared in the late Triassic.

On land, major extinctions occurred among the insects (35 families lost), the freshwater bony fishes (8) and the thecodontians (8). There was a major faunal turnover amongst non-marine tetrapods in the late Triassic, during which the formerly dominant labyrinthodonts, mammal-like reptiles, thecodontians and rhynchosaurs died out, or were greatly depleted, and new groups, such as the dinosaurs, crocodiles, pterosaurs, turtles, lepidosaurs and mammals, arrived on the scene^{14–18}.

Several authors have already noted that the extinctions in the late Triassic were either not synchronous in the sea and on land^{11,15}, or that the extinction lasted for much of the late Triassic, through the Carnian, Norian and Rhaetian stages⁵ (the 'Rhaetian' is included here in the late Norian stage^{11,12}). A more detailed study of events during this timespan of 18–25 Myr is therefore required.

Here I present data on the rates of extinction of families of marine and non-marine organisms during the late Triassic. Some groups of organisms whose fossil records are relatively well known are examined first, in order to establish the kinds of patterns of mass extinction that occurred in the late Triassic. Then a broader analysis of the fossil record of all plants and animals during that time is attempted.

The ammonoids—cephalopod molluscs with coiled shells—have a relatively good Triassic fossil record¹²; 35 ammonoid zones are recognized in the Triassic, which lasted for 35–41 Myr^{19–21}. Tozer¹² has summarized the stratigraphic ranges of the 75 families of Triassic ammonoids accurate to the level of zones. The numbers of families present in each zone (or substage, in the Scythian) are plotted as a simple histogram in Fig. 1A; the diagram has been extended into the early Jurassic²² in order

Table 1 Total numbers of families of marine and non-marine animals and plants present in each stratigraphic stage from the middle Triassic (Ladinian) to the early Jurassic (Sinemurian)

	Ladinian	Carnian	Lower Norian	Middle Norian	Upper Norian	Hettangian	Sinemurian
Marine families							
Protozoa	43	46	45*	45*	50*	49*	52*
Porifera	33	36	28*	28*	30*	30	30
Coelenterata	19	20	20*	21*	20*	20	21
Ctenophora	1	1	1	1	1	1	1
Priapulida	1	1	1	1	1	1	1
Nematoda	1	1	1	1	1	1	1
Mollusca							
Gastropoda	53*	51*	48*	49*	46*	47*	49*
Bivalvia	49	56	57	57	58	58	57
Cephalopoda	34	37*	23*	29*	22*	13	18
Others	6	6	6	6	6	6	6
Sipunculida	1	1	1	1	1	1	1
Annelida	21	20	20	20	21	21	21
Arthropoda	21	22†	23†	23†	20†	20*	23*
Bryozoa	4	4	5	5	6	6	6
Brachiopoda	17	18	17*	17*	18*	12	14
Chaetognatha	1	1	1	1	1	1	1
Echinodermata	16*	18	19*	19*	21*	31	33
Pogonophora	1	1	1	1	1	1	1
Conodonts	2	2	2	2	2	—	—
Chordata	18	14	8*	9*	11*	13	17
Total:	342*	356*	327*	338*	337*	332*	353*
Non-marine families							
'Thallophyta' (genera/orders)	33	33	33	33	35	39	37
Bryophyta (orders)	4	4	4	4	5	5	5
Charophyta	2	2	2	2	2	2	2
Pteridophyta	11	13	13	13	14	15	15
Gymnospermophyta	6	11	10	10	16	17	15
Mollusca							
Gastropoda	8	8	8	8	8	8	8
Bivalvia	3	4	4†	4†	3†	4†	3†
Onychophora	1	1	1	1	1	1	1
Arthropoda							
Insecta	30	35†	36†	37†	63†	55†	63†
Others	21	20	20‡	21‡	22‡	21	21
Chordata							
'Fish'	24	16*	13‡	13‡	13‡	11	11
Tetrapoda	12	23	16	21	25	18	16
Total:	155	170*	160‡	167‡	207‡	206*	197*
Overall no. of families	497*	526*	487*	505*	544*	538*	550*

The Norian is subdivided into three units which correspond to the Lower, Middle and Upper substages¹². Data are listed by phyla or other major groups from refs 27–32. Families that have both marine and non-marine representatives are counted once only as 'marine'. In some cases, the precise stage of origination or termination of a family has not been established, and estimates have been calculated on the basis of stratigraphic stage durations and an assumption of equal probabilities through time. Thus, for 'Middle Triassic' originations and terminations, half are assumed to have occurred in the Ladinian. For 'Upper Triassic' originations and terminations, one-quarter are assumed to have occurred in each of the Carnian, Lower Norian, Middle Norian and Upper Norian. For 'Lower Jurassic' originations and terminations, one-quarter are assumed to have occurred in the Hettangian and in the Sinemurian (the remainder of the Lower Jurassic is approximately equal in duration to the Hettangian plus Sinemurian). These uncertain assignments represent ~5% of the total number of families. In addition, certain family extinctions in the Norian stage have not been assigned to the Lower, Middle or Upper Norian divisions. One-third of all such extinctions are assumed to have occurred in each division. In cases where these calculations have been applied, an error estimate is indicated as follows: * < ±5%; ‡ < ±10%; † > ±10%.

to compare the late Triassic events with those that followed. Several declines in family diversity are evident, the largest occurring in the Carnian and late Norian.

Extinction rates for ammonoid families vary considerably during the Triassic and early Jurassic. Total extinction rates (Fig. 1B) show peaks in the late Scythian, the late Ladinian and the early Carnian, with smaller peaks in the Anisian and in the middle and late Norian. The plot of per-taxon extinction rates (Fig. 1C), which take account of the numbers of families present and give a measure of the probability of extinction²³, reveals peaks in the late Scythian, the late Anisian, the early Carnian and the late Norian. Thus, the late Triassic 'mass extinction' of ammonoid families was not a single event, but consisted of at least two—one in the Carnian, and a larger one 17–20 Myr later at the end of the Norian (the 'Rhaetian'). The timing and relative sizes of these two peaks are the same when different current timescales are used^{19–21}.

The fossil record of non-marine tetrapods also lends itself to this type of analysis. The ranges of tetrapod families have been compiled^{7,18,24–26} accurate to a number of informal 'substages'²⁴ that range in length from 1 to 3.4 Myr (mean ~2 Myr). The

histogram of Triassic and early Jurassic families of non-marine tetrapods (Fig. 2A) shows declines in diversity in the early and late Scythian, at the end of the Carnian, and at the end of the Norian. These declines are matched by peaks in the total extinction rate (Fig. 2B) and per-taxon extinction rate (Fig. 2C). The mass extinction of non-marine tetrapods at the end of the Carnian was apparently the larger of the two late Triassic events, the extinction rates being approximately twice those associated with the event at the end of the Norian.

The fossil records of non-marine tetrapods and of ammonoids show that at least three mass extinction events occurred in the late Triassic: at the end of the early Carnian (ammonoids: smaller event), at the end of the Carnian (tetrapods: larger event) and at the end of the Norian, at the Triassic–Jurassic boundary (ammonoids: larger event; tetrapods: smaller event).

Below, I attempt to generalize the study to include all families of late Triassic plants and animals. The stratigraphic distributions of families of marine invertebrates and vertebrates²⁷ and non-marine tetrapods^{28,29} have already been compiled. Data on families of non-marine invertebrates and fish were extracted from various sources^{30–32}, and from an unpublished compilation

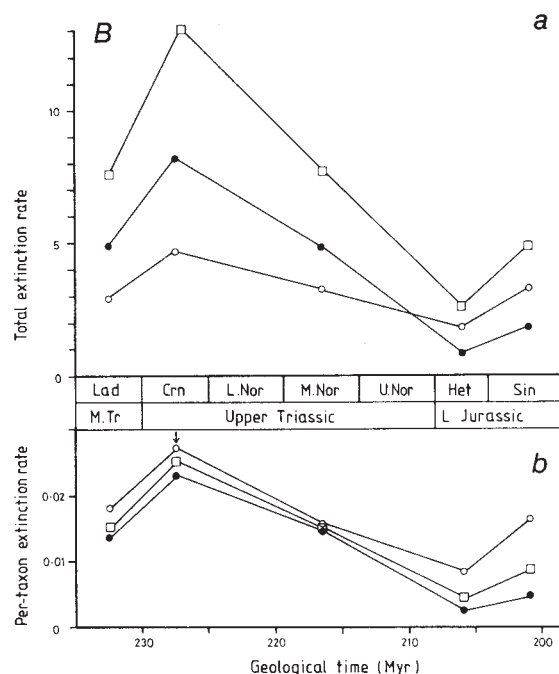
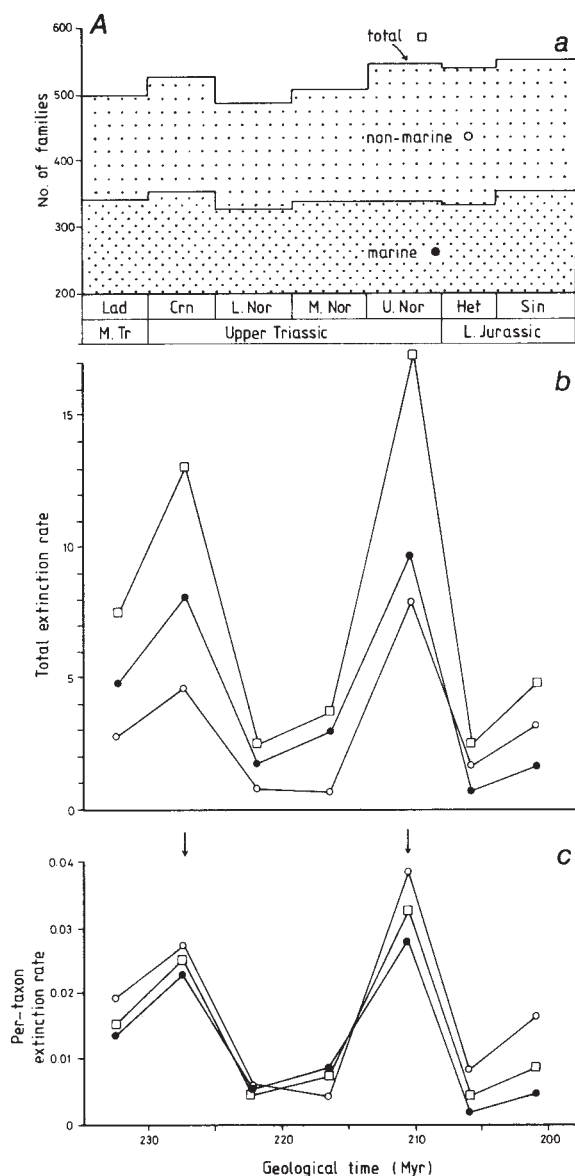


Fig. 3 **A**, Total diversity (*a*), total extinction rates (*b*) and per-taxon extinction rates (*c*) of families of late Triassic and earliest Jurassic plants and animals. The data are plotted separately for marine (●), non-marine (○) and total (□) families. The two mass extinctions (end-Carnian and end-Norian) are indicated by arrows. Data on family distributions from refs 27–32, Sepkoski's unpublished listing of non-marine families, and other sources cited therein. The data were calculated by stratigraphic stage, except for the Norian which was subdivided into Lower, Middle and Upper substages. Each Norian substage was assumed to have the same duration. **B**, Total extinction rates (*a*) and per-taxon extinction rates (*b*) of families of late Triassic and earliest Jurassic plants and animals. When the Norian data are combined, the terminal Triassic extinction event is 'smoothed out' and there appears to have been a single event in the Carnian (arrowed).

by J. J. Sepkoski Jr. Note that, because of the nature of the fossil record, the data on some groups such as insects and plants are rather poor. The time period under study spans from the Ladinian (middle Triassic) to the Sinemurian (early Jurassic), an interval of ~38 Myr²¹, and family extinctions have been noted to the level of the stratigraphic stage. The Norian stage is much longer (~17 Myr) than all the other stages (4–6 Myr), and was subdivided into Lower, Middle and Upper units, according to the standard scheme¹². The stages, and substages, used in this analysis range in duration from 4 to 6 Myr (mean 5.4 Myr).

Table 1 summarizes the diversities of families of plants and animals during the late Triassic; Table 2 summarizes the family extinctions. The present data indicate an average marine diversity of ~340 families, and non-marine diversity of ~190 families. The numbers of family extinctions per time unit range from 3 to 54 (marine; mean 23.0), and from 4 to 45 (non-marine; mean 18.1). Plots of marine and non-marine family diversity (Fig. 3Aa) show declines at the end of the Carnian, and smaller ones at the Triassic-Jurassic boundary. In all cases, both the total (Fig. 3Ab) and per-taxon (Fig. 3Ac) extinction rates show two similar peaks, a slightly higher one in the late Norian, and a lower one in the Carnian.

The two (or more) mass extinctions in the late Triassic have been identified as a single event in most previous studies^{1–4,6–10,15–17,23,28,29}. Indeed, when all the extinctions in the Norian are summed, the two mass extinction peaks are 'smoothed out' to give a single broad peak (Fig. 3B). Raup and Sepkoski^{2,33} have recently noted an extinction peak in the late Carnian in their analysis of fossil marine animal genera, but they regard it as "an artefact of sampling".

If the cyclical models of mass extinction are correct, there should have been a single event in the late Triassic, within the Norian. According to the Harland timescale¹⁹, and a 26-Myr cyclicity pattern^{1,2}, this event should have occurred at 222 Myr and, according to the Odin timescale²⁰, at 219 Myr, both in the early Norian. If it is assumed that the mass extinctions occurred at the ends of stages, the late Triassic events documented here occurred at ~225 Myr and ~213 Myr, according to the Harland timescale, at ~220 Myr and ~204 Myr, according to the Odin timescale, and at ~225 Myr and ~208 Myr, according to the 'DNAG' timescale²¹. If the preceding extinction events, at the end of the Permian and at the end of the Scythian^{1,2,5,6,16,28,29,34} (see also Figs 1, 2), are considered, the spacings between these events are highly variable, according to each of the present timescales (Table 3).

Table 2 Total numbers of extinctions of marine and non-marine families in each stratigraphic stage from the middle Triassic (Ladinian) to the early Jurassic (Sinemurian)

	Ladinian	Carnian	Lower Norian	Middle Norian	Upper Norian	Hettangian	Sinemurian
Marine families							
Protozoa	—	1	1†	—†	5†	—	—
Porifera	—	8	—	—	—	—	—
Coelenterata	—	1	—†	2†	4†	—	—
Mollusca							
Gastropoda	4	4†	2†	1†	2†	—†	1†
Bivalvia	—	1	—†	1†	6†	1	1
Cephalopoda	8	14	5†	10†	21†	1	4
Annelida	1	—	—	—	—	—	—
Arthropoda	2†	1†	1†	2†	2†	—	—
Brachiopoda	—	2	1†	1†	8†	—	1
Echinodermata	1	2	—	—	1	1	1
Conodonts	—	—	—	—	2	—	—
Chordata	8	7	—	—	3	—	2
Total:	24*	41‡	10†	17†	54‡	3†	10‡
Non-marine families							
Pteridophyta	—	—	—	—	2	—	1
Gymnospermophyta	1	—	—	—	2	2†	1†
Mollusca	—	—†	—†	1†	—†	1†	—†
Arthropoda	1	7†	5†	3†	23†	1†	5†
Chordata							
'Fish'	8	4†	—†	—†	4†	1	3
Tetrapoda	4	12	—	—	14	2	9
Total:	14	23‡	5†	4†	45‡	7†	19†
Overall no. of families	38*	66‡	15†	21†	99‡	10†	29‡

The data are listed by phyla, or other major groups, from refs 12, 18, 27–32. Stratigraphic terms and error estimates are explained in Table 1.

Table 3 Gaps (Myr) between consecutive mass extinction events in the late Permian, early Triassic and late Triassic, according to three current timescales

	Harland ¹⁹	Odin ²⁰	Palmer ²¹
Late Permian	5	6	5
Late Scythian	18	19	15
Late Carnian	12	16	17
Late Norian			

These results indicate that the record of mass extinctions is not as straightforward as has been assumed by some authors: the choice of timescale may be crucial⁵, and closer analysis of the fossil data may reveal quite different patterns from those presented so far^{1–10,23,28,29}. For example, in a recent study of the two mass extinctions in the Jurassic required by the 26-Myr cyclicity theory (end-Pliensbachian, end-Tithonian), Hallam³⁵

found that the extinctions were regional, not global, in extent, being restricted largely to Europe.

The record of mass extinctions is not yet well known. It should be possible to obtain more precise data on the stratigraphic and geographical ranges of different taxa, to refine geological timescales, and to revise the systematics of various groups. Many authors have hitherto identified a single late Triassic mass extinction but more detailed studies of particular groups (for example, ammonoids and non-marine tetrapods) and of the whole marine and non-marine fossil record have indicated that there were two quite separate mass extinction events. This conclusion suggests that recent models of bolide-mediated cyclicity of mass extinctions may be incorrect. Cladistic analyses are essential for such studies²⁹.

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A new class of Echinodermata from New Zealand

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During examination of echinoderms from sunken wood collected from depths between 1,057 and 1,208 m off the New Zealand coast, we discovered nine specimens of a small flattened discoidal invertebrate. These animals, briefly described here, superficially resemble a cnidarian medusa, but their pentamerous morphology is distinctly echinoderm-like. We consider that the features of these newly discovered echinoderms warrant the recognition of a new class. *Xyloplax medusiformis* n.gen. and n.sp. represents a radical departure in morphology from any other known extant echinoderm. Its obvious tube feet, clearly pentamerous body pattern, and calcite skeleton leave no doubt that the animal is an echinoderm. However, its concentrically arranged skeletal structures and single series of tube feet arranged in a ring are novel. The water vascular system of *Xyloplax* consists of a double ring of canals which service the tube feet in inter-radial positions; in all other living echinoderms the vascular ring (circum-oral) is single, and the tube feet are serviced from it in radial positions. These specimens represent the first new class of living echinoderms to be described since 1821. A full description of the new taxon is in preparation.

Class Concentricycloidea nov.

Diagnosis: A free-living echinoderm characterized by a weakly inflated disk-shaped body, without mouth, anus or radiating arms. Its water vascular system, including tube feet, and supporting skeletal structures are arranged concentrically on the ventral surface. The water vascular canals form a double ring with inter-radial connections to the tube feet; the ventral surface is covered by a complete velum.

Etymology: *concentricus* and *cylus* are Latin for 'concentric' and 'ring', respectively, alluding to the double water vascular ring and the concentric skeletal structures.

Order Peripodida nov.

Family Xyloplacidae nov.

Diagnoses: The same as for class.

Etymology: *peri* and *podos* are Greek for 'around' and 'foot', referring to the nearly circumferential arrangement of tube feet.

Xyloplax n.gen.

Type species: *Xyloplax medusiformis* n.sp.

Diagnosis: Body medusiform, dorsally plated with peripheral, excavate spines and uniserial, nearly circumferential tube feet.

Xyloplax medusiformis n.sp. (see Figs 1–4)

Etymology: *Xylos* and *plax* are Greek for 'wood' and 'plate' and allude to the habitat and plating of this animal; *Medusa* and *form* are Latin and allude to the animal's superficial resemblance to a cnidarian medusa.

Type material: In the National Museum of New Zealand (NMNZ) and the Australian Museum (AM). The holotype (NMNZ 4240) was collected from 42°58.6' S, 168°21.9' E to 42°57.7' S, 168°22.6' E, west of Hokitika, South Island, at a depth of 1,142–1,147 m, on water logged wood, 9 July 1983. There are eight paratypes: four (one sectioned) (NMNZ 4239) collected from 41°09.9' S, 176°26.5' E off Castlepoint, North Island, at a depth of 1,174–1,208 m, on waterlogged wood, 4 April 1984; two (one sectioned) (NMNZ 4241) from 41°18.0' S, 176°18.3' E off Castlepoint, 1,170–1,152 m, 17 October 1984; one (NMNZ

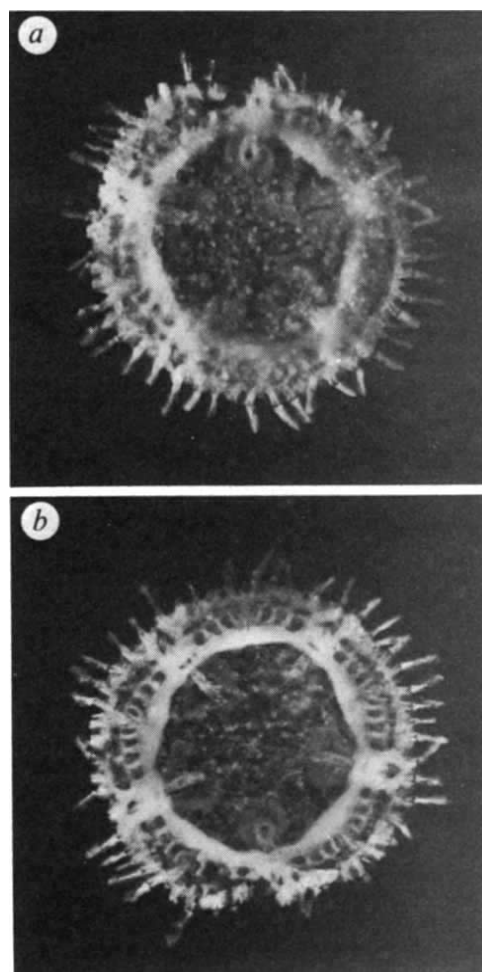


Fig. 1 Holotype (NMNZ 4240) of *Xyloplax medusiformis* n.g. and n.sp. Dorsal (a) and ventral (b) views. Diameter 3.1 mm.

4242) from 41°25.3' S, 176°07.6' E off Castlepoint, 1,110–1,057 m, on waterlogged wood, 26 May 1985; one (AM J19660) from 41°24.7' S, 176°08.4' E off Castlepoint, 1,103–1,071 m, on waterlogged wood, 16 October 1984.

Description: The following description and figures are based on the type series, four of which had to be sectioned or dissected to obtain the required information. Our terminology must be regarded as provisional at this stage.

The specimens range from 2.1 (2.6) to 7.8 (9.0) mm in diameter (including peripheral spines). The animal is circular or slightly sub-pentagonal, and weakly inflated. The dorsal surface comprises numerous finely perforated, scale-like plates, which bear small, fluted spinelets. In each of the five inter-radial is a large primary plate, the one in inter-radius CD having a hydropore that pierces the plate. The margin of the disk is fringed by small (0.6 mm) excavate spines (Figs 1, 2), and is formed by a single ring of marginal plates interrupted by five large radial plates corresponding to the terminals or oculars of asteroids.

The ventral surface is dominated by a ring of thick skeletal ossicles, the adambulacral and marginal plates (Figs 1b, 2b). The 'intramarginal' ring comprises ossicles arranged in a pentamerous pattern. Each group of ossicles has four to six elements, the medial pair (each side of the inter-radial line) being the largest. Additional ossicles are developed at the radial positions during growth.

The water vascular system forms a double ring (Fig. 3). The inner water ring runs first in a groove and then in an enclosed duct along the inner side of the ring of ossicles. The 'radial'

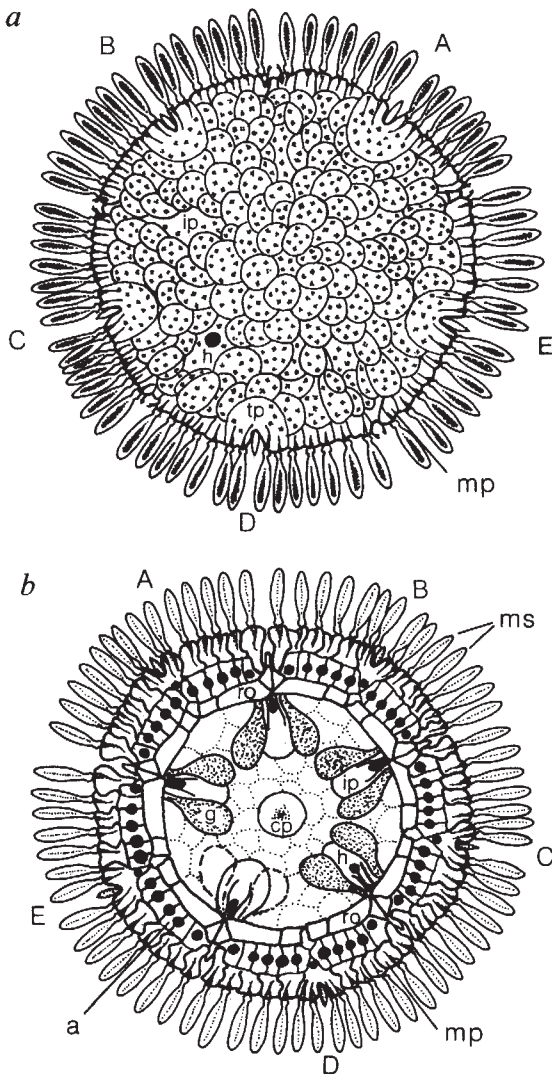


Fig. 2 Semi-diagrammatic representation of paratype NMNZ 4241 of *Xyloplax medusiformis* n.g. and n.sp. Dorsal (a) and ventral (b) views. Diameter 6.2 mm. A-E, radii; ip, Primary inter-radial plate; mp, marginal plate; tp, terminal plate; h, hydropore; ms, marginal spines; g, gonad; ro, ring ossicle; a, adambulacral plates. cp, central dorsal plate.

water canals (now outer circumferential) run along a lower, totally exposed groove on the outer side of the ring ossicles and connect with the inner ring at *inter-radial* positions. The 'radial' canal connects with each tube foot below the ampulla (Fig. 4). The tube feet are arranged in a uniserial, circumferential system, just inside the ventral margin of the disk (Figs 1b, 2b, 4).

There is no gut or mouth: the ventral surface of the animal comprises a complete and thin velum attached to the lower ridge of the ring ossicles below the groove carrying the 'radial' nerve and water canal (Fig. 4). There are five pairs of gonads or bursae which contain embryos at various stages of development, up to plated juveniles, which are similar in form to the adults.

The development of the inner water vascular ring and its protection in a groove/duct within the ossicles of the ring would not, in our view, be evolutionarily difficult to achieve. However, to meet the requirements of the new, circumferential arrangement of tube feet and the obliteration of any prolonged rays, medial splitting of the circum/radial structures, including the water canal (and presumably the nerve and haemal systems—not yet determined), must have occurred. This split could have originated as a lacuna at the junction of the circum-oral and

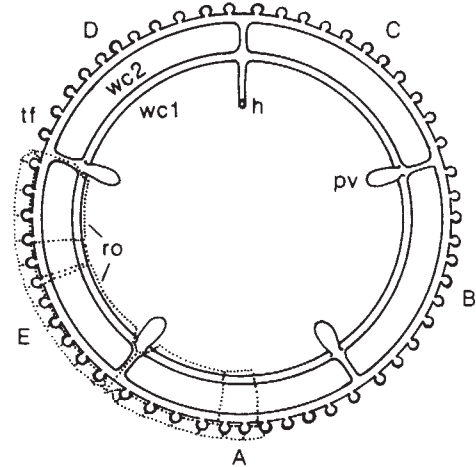


Fig. 3 Diagrammatic representation of the water vascular system of *Xyloplax medusiformis* n.g. and n.sp., determined from histological sections and by dissection. pv, Polian vesicle; tf, tube foot; wc1, circum-oral water canal; wc2, 'radial' water canal. Other abbreviations as for Fig. 2.

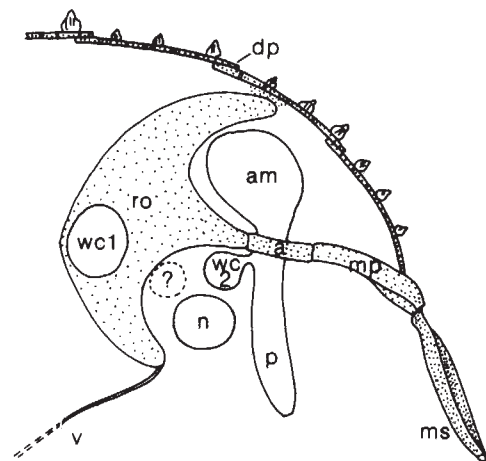


Fig. 4 Semi-diagrammatic section through the margin of *Xyloplax medusiformis* n.g. and n.sp., determined from histological sections and by dissection. am, Ampulla; dp, dorsal plate bearing fluted spinelets; n, 'radial' nerve; p, podium; v, velum; ?, 'radial' haemal canal. Other abbreviations as for Figs 2, 3.

radial canals. The lacuna could then have extended laterally and radially, stopping adjacent to the polian vesicles and terminal tube foot, respectively, and resulting in the water vascular system forming a double ring, with the connection between the two rings becoming inter-radial.

We suggest that the ossicle ring is derived from fusion of the first adambulacral/oral plate with the first two or three ambulacral plates. The tube feet must then have moved sideways and are now situated between the remaining adambulacral plates. The latter have been modified to take over the function previously fulfilled by the ambulacra. The whole inter-radial mouth frame (ossicle ring) has moved outwards, splitting the ambulacra and taking the associated circum/radial structures with it. The pivotal points are the terminal point of each radius; these points remain fixed so that a disk-like body form is derived.

We propose that the ventral velum of *Xyloplax* derived from the stretching of a simple, sac-like stomach from its original internal position, by opening of the oral frame. Thus, the outer surface of the velum is formed from the inner lining of the stomach.

Only two other classes of echinoderms have been described which have flattened, discoidal or medusiform bodies: Camptostromatoidea (Lower Cambrian) and Cyclocystoidea (Middle Ordovician–Middle Devonian). Recent studies have shown, however, that the camptostromatids do not have a medusiform body¹. The cyclocystoids do have a body form that is superficially similar to that of *Xyloplax*; a comparative discussion will be published elsewhere.

We believe that *X. medusiformis* is likely to move from niche to niche using a parachute-like mode of locomotion in bottom-water currents.

We include the Class Concentricycloidea within the subphylum Asterozoa, rather than the Crinozoa or Echinozoa; this is justified on the grounds of pattern of growth, which occurs in typical asterozoan fashion². Also, the tube feet occur between, not through, plates and on the underside of the animal. The diagnosis for the Asterozoa will need to be expanded to accommodate the special features of these new echinoderms, which we are also unable to classify within the only other asterozoan Class Stelleroidea². Our discovery supports current views^{3–5} that the present higher classification of the Echinodermata requires revision.

We thank the following colleagues for assistance: Mr Bruce Marshall, National Museum of New Zealand, sorted the echinoderms from sunken wood and discussed the habitat with us; Professor D. T. Anderson, University of Sydney, made valuable suggestions during the interpretation of this animal and arranged for Ms Kate Jakes to prepare histological sections; Dr Stephen Clark, Nelson Hospital, sectioned and photographed the tube feet; and Wim Spiekman, National Museum of New Zealand, made scale models of the ring ossicles. F.W.E.R. thanks the Marine Sciences and Technology Committee, Australia, for support. A.N.B. thanks the Zoology Department, Victoria University of Wellington, for the use of photomicrographic facilities.

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Benzodiazepine impairs and β -carboline enhances performance in learning and memory tasks

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Benzodiazepines are widely used anxiolytics and anticonvulsants, and their potent sedative properties are routinely used in presurgical anaesthesia. However, they are also known to induce a strong anterograde amnesia in patients¹. Specific benzodiazepine antagonists have recently been described^{2,3}, some of which have intrinsic pharmacological properties that are opposite to those of benzodiazepines. These have been called inverse agonists^{4,5} and they have been shown to be proconvulsant or convulsant^{6,7} whereas benzodiazepines are anticonvulsants. Inverse agonists are also anxiogenic^{8–12} rather than anxiolytic. Since benzodiazepines induce anterograde amnesia, we have investigated the possibility that inverse agonists might also have an opposite effect for this property and so enhance acquisition (learning) and (or) retention (memory). We report here that, in three different animal models, an inverse agonist of the β -carboline group, methyl β -carboline-3-carboxylate (β -CCM), enhances animal performance in three different tasks used to investigate learning and memory.

The first model, habituation to a new environment in rodents, has been used previously to demonstrate the amnesic properties of benzodiazepines¹³. The second model, a passive avoidance task, has been widely used in the study of memory processes in rodents. Finally, to investigate the effects of β -CCM in a very different learning situation, a third model, imprinting in chicks, was used¹⁴. Imprinting, a phenomenon first described by Konrad Lorenz involves newly hatched birds which are apparently more 'naive' than adult rodents. The effects of β -CCM were compared with those of diazepam, a typical benzodiazepine.

In the habituation model, food-deprived mice were placed in an unfamiliar environment (a different cage) with food *ad*

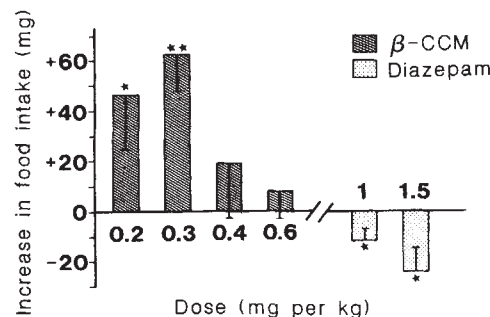


Fig. 1 Effect of β -CCM and diazepam on retention of habituation to a new environment in mice. The results show differences ($\pm$ s.e.) in the food intake of drug-treated groups compared with saline groups. Hence, ordinate 0 corresponds to the mean of the food intake of saline-treated animals during the 2 min of the test (25 ± 4 mg, $n = 60$). All groups consisted of 10 mice, except for diazepam, 1 mg per kg, where 20 mice were used. * $P < 0.05$; ** $P < 0.01$ (analysis of variance).

Methods. Swiss mice (Iffa-Credo, France, males, 30 g) food-deprived for 24 h, were injected subcutaneously with β -CCM, diazepam or saline. They were transferred to the experimental room 15 min later and placed individually for 30 s in an unfamiliar metallic cage ($32 \times 24 \times 15$ cm) containing their usual food. During this very short period, food intake was practically nil for all groups. No differences in locomotor activity could be measured among the groups; this was measured by dividing the floor of the cage into four identical squares and recording the number of times the animals crossed from one square to another during 30 s (number of crossings (mean $\pm$ s.e.): controls 2.8 ± 0.4 ; β -CCM, 0.2 mg per kg: 2.3 ± 0.4 ; β -CCM, 0.3 mg per kg: 3.6 ± 0.5 ; diazepam, 1.5 mg per kg: 2.1 ± 0.5). Following this training session, mice were returned to their polystyrene home cage in the animal quarters with no food. Food was provided *ad libitum* at the end of the day. Three days later the mice were once more deprived of food. After fasting for 24 h, they were again placed in the metallic cage for a 2-min test session. Food intake was measured.

libitum. During this training session, they explored the new cage, but ate only a small quantity. When placed in the same environment for a test session a few days later, the same food-deprived animals ate much more than during the training session. This increase in food intake is assumed to indicate that during the

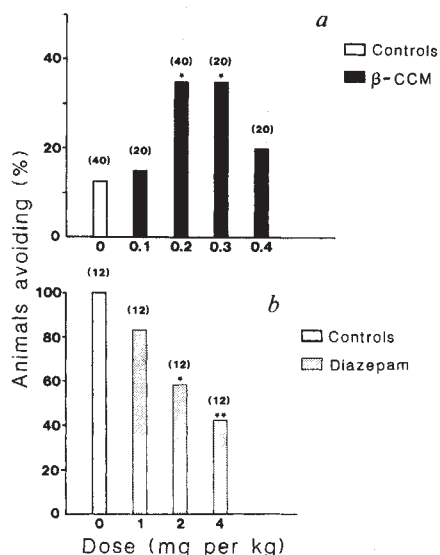


Fig. 2 Effects of β -CCM and diazepam on retention of a passive avoidance task in mice. The number of animals per group is shown in parentheses. * $P < 0.05$; ** $P < 0.01$ between treated and control animals (χ^2 test).

Methods. Swiss mice (Charles River, France, males, 25 g) were injected with saline, β -CCM or diazepam. After 15 min (saline, β -CCM) or 30 min (saline, diazepam), the animals were placed in an illuminated box ($10 \times 10 \times 12$ cm) connected to a large dark box ($23 \times 16 \times 12$ cm) that had an electrified metallic grid floor. When the mouse stepped into the dark box, a footshock was delivered to its paws. In experiments with β -CCM, where facilitation of retention was investigated, a low shock (0.1 mA for 2 s, unscrambled d.c. current) was delivered. In experiments with diazepam, where impairment of retention was investigated, a high shock (0.6 mA for 2 s, unscrambled d.c. current) was used. Latency to enter the dark box did not differ significantly during the training session, under the influence of the drugs (mean in s, $\pm$ s.e.; β -CCM controls: 9.8 ± 1.0 ; β -CCM, 0.2 mg per kg: 8.8 ± 1.1 ; β -CCM, 0.3 mg per kg: 10.8 ± 1.1 ; diazepam controls: 10.5 ± 0.9 ; diazepam, 2 mg per kg: 15.5 ± 2.6 ; diazepam, 4 mg per kg: 16.3 ± 4.2). During the test session 24 h later, mice were again placed in the lit box. Mice avoiding the dark compartment for over 60 s were considered as remembering the task. In a control experiment, shock was not delivered in the dark box. Instead, after stepping in the dark box during the first session, mice were returned to their home cages for 5 min; they were then shocked in a different lit box, thus dissociating dark box from punishment. 24 h later, during the test session, no difference was observed between saline and treated subjects. Percentages of animals ($n = 15$ per group) avoiding the dark box were: saline: 8%; β -CCM, 0.2 mg per kg: 0%; β -CCM, 0.3 mg per kg: 17%. This control experiment rules out a nonspecific effect of β -CCM on avoidance of the dark box.

training session the animals become accustomed to the new environment and that during the test session, they remember it.

In our model, when β -CCM (0.2 and 0.3 mg per kg body weight) was administered to mice before the training session, food intake during the 30 s of the session did not differ from that of controls or diazepam-treated animals. When the same animals were tested 4 days later, by which time all drugs would have been eliminated⁷, food intake of the β -CCM group was enhanced compared with that of controls. While the average consumption of the controls was 25 mg during the 2-min test session, the β -CCM group consumed 40–60 mg more (Fig. 1). This effect of β -CCM injected only before the training sessions disappeared when higher doses (0.4 and 0.6 mg per kg), close to those we have shown previously¹¹ to be anxiogenic doses (1 mg per kg), were used. On the other hand, when diazepam

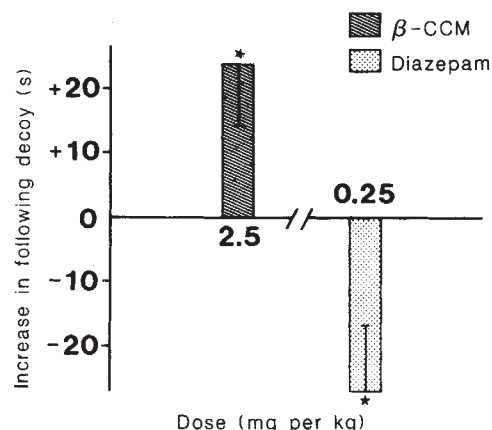


Fig. 3 Effect of β -CCM and diazepam on retention of imprinting (following a ball) in chicks. Results are the differences ($\pm$ s.e.) in the time the drug-treated chicks spent following the ball compared with saline-treated groups. Hence, ordinate 0 corresponds to the mean of the time spent following the ball by saline-treated chicks (54 ± 8 s, $n = 56$); $n = 17$ and 18 for β -CCM-treated and control groups, respectively; $n = 39$ and 38 for diazepam-treated and control groups, respectively. * $P < 0.025$ between treated and controls (analysis of variance).

Methods. Chicks (24 h old, Hubbard strain, Bas-Bourray, France) were injected i.p. with saline, β -CCM or diazepam, 5 min before imprinting. The imprinting session was performed as described previously¹⁴. Briefly, the chick was placed for 5 min in a black circular arena (200 cm diameter) facing a decoy (red ball, 11 cm diameter, with yellow stripes). The ball was suspended 5 cm above the floor and moved at a speed of 385 cm min^{-1} . The time the chick spent following the ball was recorded automatically. During this 5-min imprinting session, the average time was 24 ± 3 s with no significant difference between treated subjects and their controls (increase in following behaviour (s) compared with controls $\pm$ s.e.: β -CCM, 1.88 ± 6.22 ; diazepam, 6.04 ± 6.47). 24 h later, a 5-min retention test was performed in the same apparatus.

(1.0 and 1.5 mg per kg) was administered before the training session, a reduction in food intake was seen during the test session (Fig. 1).

In the passive avoidance test, mice were trained in a single-trial procedure. The animals were placed in an illuminated box connected to a dark box. Entrance into the dark box usually occurred within 30 s and was punished by an electric footshock. On the next day (test session), the same mice were again placed in the illuminated box. Mice staying in the illuminated box for more than 60 s were considered as remembering the task. Retention was thus quantified by the percentage of animals avoiding the dark compartment.

When facilitation of retention was to be investigated, a low-intensity shock was delivered; when impairment of retention was to be investigated, a high-intensity shock was used. In the low-shock group (Fig. 2a), controls showed a poor level of retention and, during the test session, only a small percentage (12%) of subjects avoided the dark box. In this low-shock group, when β -CCM (0.2 and 0.3 mg per kg) was administered before the training session, a much higher percentage (35%) of the animals now avoided the dark box during the test session. Lower (0.1 mg per kg) and higher (0.4 mg per kg) doses of β -CCM were less effective. Hot-plate tests and measurement of latencies of response to shocks showed that β -CCM had no effect on pain threshold¹¹. The enhancement of performance by β -CCM in this task is in direct opposition to the amnesic effect of diazepam. Thus, in the high-shock group the percentage of mice avoiding the dark box during the test session was reduced from 100% (controls) to nearly 40% (diazepam-treated) (Fig. 2b).

For the imprinting experiments, newly hatched chicks (~ 24 h

Table 1 Effects of β -CCM and diazepam on performance during a passive avoidance test 30 s after training

Test substance	% Of animals avoiding dark box during test session
Low shock	
Saline	10
β -CCM (0.3 mg per kg)	40*
High shock	
Saline	87
Diazepam (2 mg per kg)	27†

The protocol was the same as for Fig. 2, except that the test session was performed 30 s, instead of 24 h, after the end of the training session ($n = 20$ and $n = 15$ for high- and low-shock groups, respectively).

* $P < 0.05$; † $P < 0.001$ between treated and control animals (χ^2 test).

old) were used. Chicks tend to follow a moving decoy presented to them during the imprinting session. When retested in the same situation 24 h later, imprinted chicks, having memorized the characteristics of the situation and of the decoy, follow the decoy for a longer time than do non-imprinted chicks.

In our experiment, when imprinting was accomplished under the effect of β -CCM (2.5 mg per kg), the time spent by the chick in following the decoy (a ball) during the test session was increased compared with untreated controls (Fig. 3). Thus, in the 5-min period of the test session, controls followed the ball for an average 53 s whereas β -CCM-treated chicks followed it for about 20 s longer. On the other hand, when imprinting was performed under the effect of diazepam (0.25 mg per kg), the time spent following the ball during the test session decreased; treated subjects followed the ball for about 20 s less than controls.

Our results from these three different animal models suggest that β -CCM administered before acquisition enhances the performance of the task during the retention test. We verified that such an effect cannot be obtained when the drug is administered immediately after acquisition or before the retention test. For instance, in the study of habituation to a new environment, when β -CCM (0.3 mg per kg) was administered just after the training session, there was no significant difference in the food intake of these animals during the test session compared with the controls (difference of food intake with the controls -1.3 ± 16.24 mg). Similarly, if β -CCM (0.3 mg per kg) was administered 15 min before the test session, the difference in intake of the treated group as compared with the controls remained non-significant (difference -15.7 ± 19.92 mg).

In contrast to the improvement of retention when β -CCM was administered before acquisition, diazepam had the opposite effect and, in agreement with previous reports, impaired the performance during the retention test. Such an impairment has been interpreted previously as a demonstration of the amnesic effect of diazepam¹. However, it has also been suggested that the effect of diazepam is due to 'state dependency'¹⁵. According to this hypothesis, when learning occurs under the effect of a drug, proper retrieval is achieved only in the presence of the same drug. This hypothesis was tested by reinjecting some of the diazepam-treated subjects from the three models with diazepam before the retention test. No improvement in performance was seen in the three models. The state-dependency hypothesis would thus not explain the impaired performance of diazepam-treated subjects. Our results support the idea that diazepam impairs memory processing, a result consistent with studies using human patients¹. As β -CCM has opposite effects to those of diazepam, it could be considered as enhancing memory processing. These effects of β -CCM were not seen in the dose range of the anxiogenic or convulsant effects of the drug, as the optimum dose in the present study (0.2, 0.3 mg per kg) is much lower than the anxiogenic (1 mg per kg)¹¹ or convulsive (1–10 mg per kg)⁷ doses in the same strain of mice.

To investigate whether these effects of β -CCM and diazepam are mediated by the benzodiazepine receptor, we attempted to block their actions by using the specific benzodiazepine antagonist Ro 15-1788 (refs 2–3). This experiment was undertaken in the passive avoidance situation described for Fig. 2 and with the same protocol. Thus, low- and high-shock groups were used to study the effects of β -CCM and diazepam, respectively. Groups of mice were injected, before the first session, with β -CCM (0.3 mg per kg subcutaneously (s.c.)), diazepam (4 mg per kg intraperitoneally (i.p.)) or Ro 15-1788 (15 mg per kg, i.p.) or a combination of β -CCM + Ro 15-1788 or diazepam + Ro 15-1788. Results show that Ro 15-1788 had no effect when administered alone. However, the effects of β -CCM or diazepam were completely antagonized by co-administration of Ro 15-1788. This indicates that both the effect of β -CCM and the opposite effect of diazepam are mediated by the benzodiazepine receptor.

The next question was to delineate which stages of learning or memory were being affected by the drug. If memory can be defined as the retention of information, this information needs to be acquired (learning) and eventually recalled (retrieval). Since in our experiments, drugs have no effect when administered just before the retention test, we can rule out an action on retrieval. This agrees with most results obtained with benzodiazepines in human subjects¹. A possible explanation of the data would be that β -CCM increases the level of arousal during the training session. This explanation would agree with the observation that arousal-enhancing drugs improve learning¹⁶ and that several β -carbolines increase arousal¹⁷. We tried to confirm this interpretation by using the passive avoidance situation described earlier, the only difference being that the test session was performed 30 s, instead of 24 h, after the end of the training session. The results were the same as with a 24-h interval (Table 1), suggesting an effect on learning rather than memory. Our results with β -CCM could therefore be interpreted as an improvement of performance due to arousal-induced improvement of learning. Conversely, benzodiazepines could act through a reduction of arousal during the training session. However, the effects of benzodiazepines on short-term memory in humans suggest that benzodiazepines not only decrease acquisition (learning) but also seem to interfere with retention (memory). Finally, while it is likely that the observed effects with β -CCM and diazepam are related to the acquisition side of memory processing, the precise mechanisms underlying these effects remain unknown.

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The structural gene coding for myelin-associated proteolipid protein is mutated in *jimpy* mice

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Mutations affecting developmental processes may allow some insight into the complexity of the biological processes involved. In mice, two mutants that affect myelin formation in the central nervous system, *jimpy* and *shiverer*, have proved to be useful models for the study of this process¹. The predominant proteins in myelin are the major myelin proteolipid (PLP) and the myelin basic proteins (MBP), which together account for 80–90% of total myelin proteins². It has recently been shown that the *shiverer* mutation is located in the MBP structural gene^{3,4}, but the site of the *jimpy* mutation, which is X-chromosome-linked and may be similar to the sex-linked dysmyelination human disease, Pelizaeus–Merzbacher disease⁵, remains unclear. Here we provide evidence, based on a combined genetic and biochemical approach, that the sex-linked recessive mutation *jimpy* is located in the structural gene coding for PLP.

P-23, a complementary DNA probe corresponding to a PLP gene transcript, has recently been cloned from a rat brain-specific cDNA library using an oligonucleotide probe corresponding to amino acids 231–235 of bovine PLP⁶. This probe has subsequently been shown to react not only with rat DNA but also with human and mouse DNAs. Southern blotting analysis of *Eco*RI-restricted DNAs from a panel of mouse–hamster somatic cell hybrids, using P-23, revealed a single band whose distribution indicated that the sequence recognized by probe P-23 was on the mouse X chromosome (data not shown). There was no evidence for the presence of crossreacting sequences on other chromosomes.

To localize the PLP gene more precisely and to provide further data to allow correlation of the cytological and genetic X-chromosome profiles, the position of the gene was analysed by *in situ* hybridization and an interspecific mouse cross.

In situ hybridization experiments with probe P-23 were carried out using metaphase spreads from a female WMP mouse, in which all chromosomes other than chromosomes X and 19 are in the form of metacentric robertsonian translocations. From 50 metaphases analysed 55 grains were scored on the X chromosome; 69% of these grains mapped to the F chromosome band (Fig. 1a), with a maximal distribution in the proximal part of this band, suggesting that the PLP structural gene maps to XF1 (Fig. 1b).

Genetic localization was facilitated when it was found that the P-23 probe detected an *Eco*RI restriction fragment length polymorphism (RFLP) between a B6.CBA.RI strain of the *Mus. musculus domesticus* species and the inbred SPE/Pas strain derived from *Mus. spretus* (Fig. 2), thereby raising the possibility of using an interspecies backcross between the two strains (for details see Fig. 3 legend) to localize the PLP structural gene. Meiotic recombinants from this backcross segregated not only for the X-linked *Tabby* (*Ta*), *jimpy* (*jp*) and *hypoxanthine phosphoribosyltransferase* (*hprt*) markers but also for RFLPs corre-

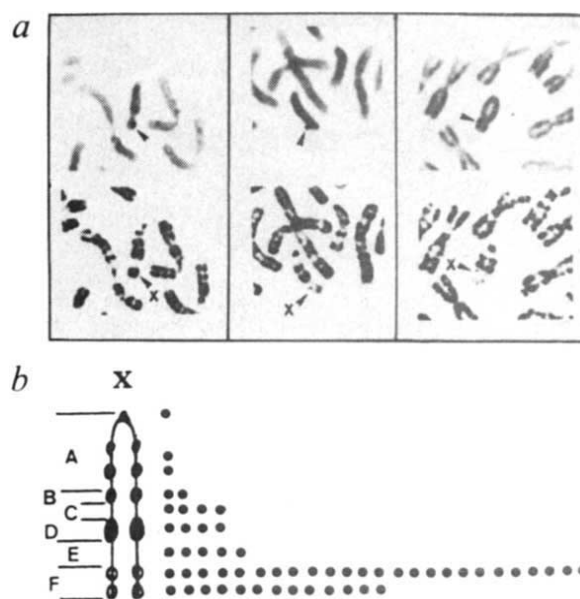


Fig. 1 Localization of the rat PLP probe to the mouse X chromosome by *in situ* hybridization. The pBR322 plasmid containing the P-23 PLP cDNA clone was used to screen R-banded WMP female mouse metaphase preparations using standard procedures¹⁴. a, Ultraviolet light image (upper) and normal light image (lower) of a portion of one such metaphase spread. The arrows indicate grains at position XF1. b, Composite grain distribution pattern of the ³H-PLP probe on R-banded metaphase spreads; ●, grains per band. A–F represent the chromosome bands (see ref. 15 for banding nomenclature).

sponding to six X-linked anonymous unique sequence probes⁷ and three X–Y common sequences (P.A. and C. Bishop, in preparation). Figure 3a shows the localization of the markers most relevant to the present study.

The hybridization profile of backcross progeny from this cross (Fig. 2) confirmed that the probe recognized a restriction fragment in the X chromosome because: (1) a dosage effect was noted when female and male progeny were observed; and (2) whereas male progeny never showed more than one restriction fragment, 57% of female progeny showed a doublet, indicating the simultaneous presence of the two allelic forms. Typing of 90 such backcross progeny with the PLP cDNA probe enabled the gene coding for this sequence to be localized using two approaches; first, by inspection of the pedigrees of the various animals, allowing correlation between the crossover events that occur on the X chromosome of each animal and their PLP haplotype; and second, by estimation of recombination frequencies in the different intervals defined by the markers *hprt*, *Tabby*, 45 and 52 (see Fig. 3a).

Figure 3b shows the former approach for a limited number of female and male animals. For example, examination of animals 4, 12, 14, 18, 33, 37, 44, 55 and 67 suggests that the PLP gene (*plp*) cannot lie in the region represented by probe 100 between the centromere and *hprt*. Similarly, results with animals 4, 12, 33, 37, 44, 55 and 67 suggest that the PLP gene cannot be proximal to the *Tabby* gene. Overall, the pedigree analysis indicates that the PLP gene must lie between markers 52 and 45, a region comprising some 26 centimorgans and including the *jimpy* locus⁷.

Examination of the recombination frequency data obtained from female progeny is in complete agreement with the above conclusions. The recombination frequencies obtained were: *hprt*–*plp*, 34%; *Ta*–*plp*, 20%; 52–*plp*, 21%; and 45–*plp*, 17%. Thus, the *plp* locus is some 20 centimorgans distal from *Tabby* and 34 centimorgans distal from *hprt*. This latter value is exactly

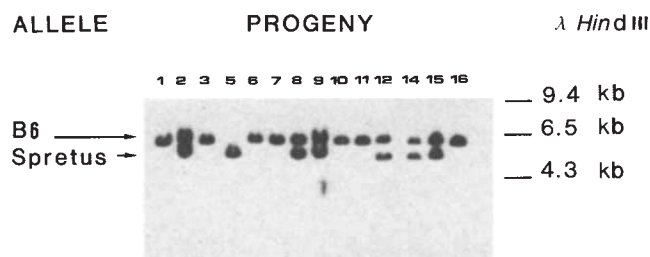


Fig. 2 Progeny haplotype characterization using the PLP P-23 insert on *EcoRI*-restricted genomic DNAs. Washing conditions were $0.2 \times \text{SSC}$ and 65°C . The numbers identify individual backcross mice progeny. kb, kilobases.

that reported for the distance between *hpri* and the *jp* mutation⁸ suggesting that the PLP probe is detecting a locus either identical to or closely linked to *jimpy*. Support for this was obtained by the PLP probe and associated with the B6.CBA.RI strains, although the number of such progeny tested is small (eight animals).

No evidence for the presence of a sizeable deletion within the PLP gene was detected in *jimpy* mice by Southern blot analysis. Probing of genomic blots restricted with a panel of enzymes including *HindIII*, *EcoRI*, *PstI*, *BglI* and *MspI* has failed to reveal a RFLP associated with the *jimpy* mutation (data not shown). In view of this and the fact that even the analysis of very large numbers of such backcross progeny cannot exclude very tight linkage rather than gene identity, the PLP cDNA probe was assumed, on the basis of the mapping data, to be the product of the *jimpy* gene. We therefore attempted to confirm this by using Northern, dot-blot and S_1 mapping techniques with probe P-23 to compare populations of messenger RNA molecules extracted from the brain of male *jp/Y* and female *jp/+* animals and control male $+/Y$ and female $+/+$ progeny. No significant difference in overall brain mRNA levels was noted between the different classes of animals. However, quantitative differences in the level of mRNA complementary to P-23 were clearly evident from both Northern blot and dot-blot experiments. The Northern blot in Fig. 4a shows that P-23 hybridizes to two classes of mRNA transcripts in $+/+$ females and $+/Y$ males which are respectively 3,200 and 2,400 base pairs (bp) long; the smaller transcript is considerably less abundant than the larger one. The presence of two PLP transcripts in normal mouse mRNA⁹ and in rat brain has been reported previously^{9,10}. Both transcripts are present in heterozygous *jp/+* females, although in much reduced quantity. In *jp/Y* males these are small amounts of a transcript of similar size to the larger 3,200-bp transcript, the 2,400-bp transcript being either absent or, more probably, present in only trace amounts.

RNA dot hybridization experiments using the P-23 probe (Fig. 4b) show that brains of heterozygous *jp/+* females contain 60–70% of the PLP transcripts present in $+/+$ females. Brains of male *jp* mice, on the other hand, contain only 15–20% of the PLP mRNA levels found in control males. The slight quantitative discrepancy between the dot-blot and Northern blot experiments over the quantity of RNA in *jp/Y* male hybridizing with the PLP probe may be due either to differential sensitivity linked to the use of poly(A)⁺ RNA rather than total RNA for the dot-blot experiments, or to accumulation of precursor mRNAs detected only in the latter analysis.

Proof that it is the PLP structural gene that is mutated in *jimpy* mice was provided by S_1 nuclease protection experiments using poly(A)⁺ brain RNA from *jimpy* and normal wild-type mice. Figure 4c shows the results obtained using a probe corresponding to the 5' end of the PLP mRNA molecule. Two main differences were observed between mutant *jp/Y* male and normal male ($+/Y$) brain mRNAs. Whereas both $+/Y$ male and

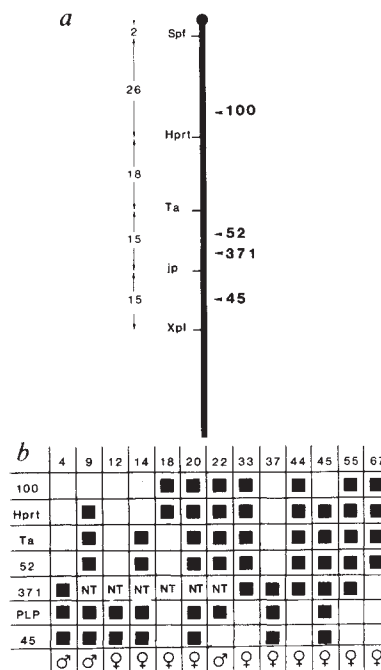


Fig. 3 Pedigree analysis of the R1 RFLP in B6.CBA.RI $\times$ Spe/Pas backcross progeny, detected by the PLP P-23 cDNA. *a*, Map localization of the mouse X chromosome markers used in the present study. Localization of molecular probes 100, 52 and 45 is detailed in ref. 7. Details of the localization of the X-Y common 371 probe will be reported elsewhere (P.A. and C. Bishop, in preparation). *b*, Distribution of RFLPs of the *Mus. spretus* type (■) and B6.CBA.RI type (□) detected in individual interspecies backcross progeny using molecular probes 100, 52, 45, and P-23 (*plp*), HPRT allozymic forms and the *Tabby* coat colour marker. The interspecies backcross used was set up by crossing female B6.CBA.RI mice (*hpri^b Ta jp/hpri^b++*) with male Spe mice (*hpri^a++/Y*). Resulting F₁ females (*hpri^b Ta jp/hpri^a++* or *hpri^b Ta+/hpri^a++*) were backcrossed to B6.CBA.RI males (*hpri^b++/Y*) and progeny analysed for the *Tabby* and *jimpy* markers. HPRT analysis, DNA extraction, restriction blotting and probing of genomic blots was as described in ref. 7. Only the X chromosome derived from the F₁ female is illustrated. In the region between the centromere and *hpri*, for reasons of clarity, only one of the four possible typed markers has been represented. NT, not tested.

jp/+ female mouse brain mRNAs protect the probe over a region of some 800 nucleotides, protection by mRNA from *jimpy* mice (*jp/Y*) extends only over ~630 nucleotides, indicating an apparent loss of S_1 protection over a region of some 160 nucleotides in the *jimpy* PLP mRNA molecule compared with the wild type. It is unclear whether the 160-nucleotide band seen only in the *jp/Y* track represents this sequence, but if it does, the deletion occurring in *jimpy* mice must be very limited in extent. The second difference observed with brain mRNA from *jimpy* mice is its failure to protect the 280-nucleotide band otherwise found in both $+/Y$ male and $+/jp$ female mRNA tracks. Furthermore, there was no detectable difference between the *jimpy* and wild-type S_1 profiles when a 3' non-coding PLP fragment (*Pst* 800–*Pst* 1,334)⁶ was used instead of the 5' probe (data not shown).

Although this comparative analysis confirms the presence of a mutation in the PLP structural gene, elucidation of the type of mutation which could account for the differential hybridization pattern is complicated by the presence of two supernumerary bands of 380 and 280 nucleotides in both wild-type and $+/jp$ females. These bands cannot be attributed to differences between rat and mouse *plp* sequences because the S_1 profiles

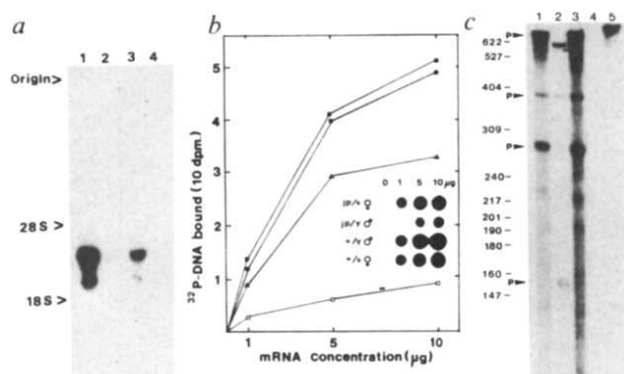


Fig. 4 *a*, Northern blot analysis of RNA from the brains of control (lane 1), hemizygous *jp/Y* (lane 2) and heterozygous *jp/+* mice (lane 3). Lane 4, purified ribosomal RNA (20 μg). The 5.1-kb and 2.0-kb positions indicate 28S and 18S mouse ribosomal RNAs, respectively. *b*, Dot hybridization analysis of PLP-specific mRNA from mouse brains. ■, Male +/Y control; ●, female +/+ control mice; ▲, heterozygous *jp/+* female; □, hemizygous *jp/Y* male. The inset shows the autoradiographic spots (2 days' exposure) used for this analysis. Control autoradiographic spots obtained using equal quantities of *Escherichia coli* transfer RNA gave no signal. *c*, S₁ protection experiment using poly(A)⁺ RNA from *jimpy* mice. The 795-nucleotide *Pst*I-*Pst*I fragment⁶ was subcloned into vector M13mp9. Radiolabelled single-stranded DNA was prepared according to Burke¹⁶. Aliquots of 60,000 c.p.m. per probe were hybridized with 0.12 μg of poly(A)⁺ RNA from male +/Y mice (lane 1), 1.4 μg of poly(A)⁺ RNA from hemizygous *jimpy* male *jp/Y* (lane 2) or 0.5 μg of poly(A)⁺ RNA from female heterozygous *jp/+* mice (lane 3), plus 10 μg of carrier yeast tRNA. Lane 4, 5,000 c.p.m. of unhybridized probe which had been digested with S₁. Lane 5, 5,000 c.p.m. of unhybridized probe that had not been S₁ digested. After digestion with S₁ nuclease, the resulting mixture was electrophoresed through 5% acrylamide/8 M urea gel. Autoradiogram exposure was at -70 °C plus intensifying screen for 8 h. Size markers are pBR322 digested by *Hpa*II. P, protected; ND, not digested.

Methods. *a*, Total cellular RNA from 3-week-old mice was prepared, electrophoresed and transferred to nitrocellulose as described elsewhere^{17,18}. Blots were prehybridized at 42 °C in 50% formamide, 5 × SSC, 5 × Denhardt's¹⁹, 20 mM sodium phosphate pH 6.8, 250 μg ml⁻¹ salmon sperm DNA, 0.1% SDS, then hybridized with nick-translated ³²P-labelled probe²⁰ in the same solution. Blots were washed four times in 2 × SSC/0.1% SDS/0.1% pyrophosphate at room temperature and twice in 0.1 × SSC/0.1% SDS/0.1% pyrophosphate for 15 min each at 50 °C, then exposed to Kodak X-Omat AR film at -70 °C for 1 day with Cronex Lightning Plus intensifying screen. *b*, Samples of poly(A)⁺ RNA (1–10 μg) were treated with formaldehyde and spotted onto sheets of nitrocellulose according to the method of White and Bancroft²¹, prehybridized and hybridized as for the Northern analysis. Individuals blots were cut out and counted in scintillation fluid and standard curves generated by plotting counts per min of probe bound per dot against μg of RNA immobilized per dot.

observed using the 5' rat cDNA probe and wild-type mouse brain mRNA are identical to those seen with rat brain mRNA (data not shown). It remains possible that this pattern is related to the presence of a mRNA coding for the DM-20 molecule, which differs from PLP by the deletion of an internal fragment of about 40 amino acids¹¹, and further experiments are in progress to test this hypothesis and to define the mutational site further. With this finding, mutations in the structural genes coding for at least two different myelin proteins have been shown to lead to myelin deficiency and severe neurological disorders⁴. In both cases, the structural gene mutation leads to lowered steady-state mRNA levels, although it is not known whether this is due to a transcriptional or post-transcriptional event. The correlation reported here between the *jimpy* mutation and the

PLP structural gene extends previous findings suggesting that *plp* is on the X chromosome^{12,13} and underlines the interest in myelin as a model system for study of gene-gene product interactions.

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Light-dependent phosphorylation of rhodopsin by β -adrenergic receptor kinase

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The structural components involved in transduction of extracellular signals as diverse as a photon of light impinging on the retina or a hormone molecule impinging on a cell have been highly conserved. These components include a recognition unit or receptor (for example, the β -adrenergic receptor (β AR) for catecholamines or the 'light receptor' rhodopsin), a guanine nucleotide regulatory or transducing protein, and an effector enzyme (for example, adenylate cyclase or cyclic GMP phosphodiesterase)^{1,2}. Molecular cloning has revealed that the β AR shares significant sequence and three-dimensional homology with rhodopsin³. The function of the β AR is diminished by exposure to stimulatory agonists, leading to desensitization⁴. Similarly, 'light adaptation' involves decreased coupling of photoactivated rhodopsin to cGMP phosphodiesterase activation^{5–7}. Both forms of desensitization involve receptor phosphorylation. The latter is mediated by a unique protein kinase, rhodopsin kinase, which phosphorylates only the light-bleached form of rhodopsin^{8–10}. An analogous enzyme (termed β AR kinase or β ARK) phosphorylates only the agonist-occupied β AR¹¹. We report here that β ARK is also capable of phosphorylating rhodopsin in a totally light-dependent fashion. Moreover, rhodopsin kinase can phosphorylate the agonist-occupied β AR. Thus the mechanisms which regulate the function of these disparate signaling systems also appear to be similar.

Phosphorylation of the β -adrenergic receptor by β ARK is almost totally dependent on agonist occupancy of the receptor, as demonstrated in Fig. 1 (compare lanes 1 and 2). β ARK is also able to phosphorylate rhodopsin in an almost totally light-dependent manner (Fig. 1, lanes 4, 5). At comparable levels

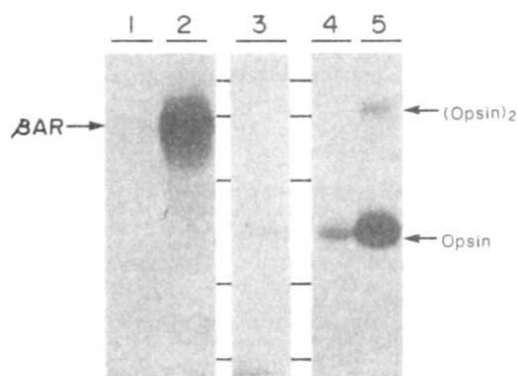


Fig. 1 Phosphorylation of the β -adrenergic receptor and rhodopsin by the β -adrenergic receptor kinase. Lane 1 contains 0.6 pmol β AR; lane 2, 0.6 pmol β AR and 20 μ M (-)-isoprenaline; lane 3, 0.6 pmol rhodopsin in the light; lane 4, 250 pmol rhodopsin in the dark; lane 5, 250 pmol rhodopsin in the light. The results shown are representative of three experiments. Relative molecular mass (M_r) standards (94,000 (94K), 67K, 45K, 30K and 20.1K) are indicated by marks between lanes 2 and 3 and between 3 and 4. **Methods.** Receptors were incubated for 60 min at 30 °C with 20 mM Tris-HCl pH 7.5, 20 mM NaCl, 5 mM sodium phosphate, 2 mM EDTA, 5 mM $MgCl_2$, 5 mM NaF, 0.05 mM [γ - ^{32}P]ATP, (2,000 c.p.m. per pmol) and 0.3 μ g β ARK in a total volume of 50 μ l. Reactions containing β AR were stopped by adding 1 ml of 100 mM NaCl, 10 mM Tris-HCl pH 7.2, 1% digitonin. After incubation for 30 min the samples were centrifuged and the supernatants desalted on Sephadex G-50 using 100 mM NaCl, 10 mM Tris-HCl pH 7.2, 0.05% digitonin. β AR was then repurified using an alprenolol affinity resin as described elsewhere¹¹. After lyophilization, 100 μ l of SDS buffer (8% SDS, 10% glycerol, 5% β -mercaptoethanol, 25 mM Tris-HCl pH 6.5) was added to each sample. Reactions containing rhodopsin were stopped by adding 50 μ l of SDS buffer. Samples were then electrophoresed on 10% homogeneous SDS-polyacrylamide gels¹⁷. Gels were dried before autoradiography for 8 h (lanes 1–3) or 15 min (lanes 4, 5). β ARK was partially purified from bovine brain by $(NH_4)_2SO_4$ precipitation (13–26%) of a high-speed supernatant fraction. The precipitate was then chromatographed on Ultrogel AcA34, DEAE Sephacel and CM Fractogel. Kinase activity was eluted with 20 mM Tris-HCl pH 7.5, 1 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride, 10 μ g ml⁻¹ leupeptin, 5 μ g ml⁻¹ pepstatin, 10 μ g ml⁻¹ benzamidine (J.L.B., F.M. Jr, R.J.L. and M.G.C., manuscript in preparation). The preparation used was ~20% pure and had a specific activity of ~35 pmol P_i per min per mg using 0.02 μ M β AR as substrate. The preparation did not contain any contaminating protein kinase C or cyclic AMP-dependent protein kinase¹¹. β AR was purified from hamster lung to >95% homogeneity by affinity chromatography and molecular-sieve HPLC as described elsewhere¹⁸. Purified β AR was reconstituted into phosphatidylcholine vesicles^{11,19} before phosphorylation. Rod outer segments (ROS) were prepared from bovine retinas by stepwise sucrose gradient centrifugation¹³. Rhodopsin kinase-free ROS were prepared by treatment with 5 M urea¹⁰ and consisted of ~95% rhodopsin, as assessed by Coomassie blue staining of SDS-polyacrylamide gels. The urea treatment effectively eliminates the endogenous rhodopsin kinase activity as rhodopsin phosphorylation in our ROS preparations was observed only in the presence of added kinase.

(0.6 pmol), β AR is a significantly better substrate for β ARK than is rhodopsin (Fig. 1, lanes 2, 3). Figure 2 shows that analogous results are achieved using rhodopsin kinase. Thus, phosphorylation of rhodopsin is almost totally light-dependent (Fig. 2, compare lanes 4 and 5) whereas phosphorylation of β AR is almost totally agonist-dependent (lanes 1, 2). In addition, while β ARK preferentially phosphorylates β AR, rhodopsin kinase phosphorylates rhodopsin better than β AR (Fig. 2, lanes 2, 3). The large apparent difference in kinase specificity, with β ARK being much better at phosphorylating β AR compared with rhodopsin and rhodopsin kinase being only slightly

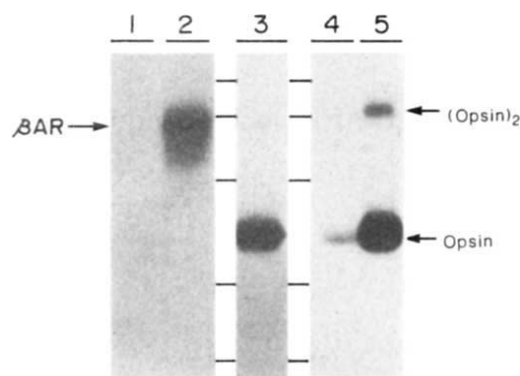


Fig. 2 Phosphorylation of the β -adrenergic receptor and rhodopsin by rhodopsin kinase. Lane 1 contains 0.6 pmol β AR; lane 2, 0.6 pmol β AR and 20 μ M (-)-isoprenaline; lane 3, 0.6 pmol rhodopsin in the light; lane 4, 250 pmol rhodopsin in the dark; lane 5, 250 pmol rhodopsin in the light. Incubations were identical to those described for Fig. 1, except that they contained 3 μ g of rhodopsin kinase instead of β ARK. β AR was repurified as described in Fig. 1 legend to remove the rhodopsin kinase autophosphorylation band which runs at M_r ~64K. Reactions containing rhodopsin were stopped by adding 50 μ l of SDS buffer before electrophoresis on 10% polyacrylamide gels. Autoradiography of the dried gel was for 24 h (lanes 1–3) or 8 min (lanes 4, 5). The results shown are representative of three experiments. M_r standards are the same as in Fig. 1.

Methods. Rhodopsin kinase was partially purified from bovine ROS extracted with 10 mM potassium phosphate, 3 mM EDTA, 2 mM EGTA pH 6.8. The high-speed supernatant from this extract was dialysed against 20 mM potassium phosphate, 0.5 mM EDTA, 0.5 mM EGTA, 20% glycerol pH 6.8 before chromatography on a Blue Sepharose column. Rhodopsin kinase activity was eluted with a KCl gradient before dialysis against 0.1 M potassium phosphate, 0.1 mM EDTA, 0.1 mM EGTA, 50% glycerol pH 6.8 (R.L.S., manuscript in preparation). The preparation used was ~10% pure and had a specific activity of ~13 nmol P_i per min per mg using 10 μ M rhodopsin as substrate. The preparation did not contain any contaminating protein kinase C or cAMP-dependent protein kinase. β AR was purified and reconstituted as described in Fig. 1 legend. Urea-treated ROS were prepared as described in Fig. 1 legend.

better at phosphorylating rhodopsin, may reflect the fact that only the bleaching intermediates metarhodopsins II and III can serve as effective substrates for rhodopsin kinase^{10,12}. Thus, the actual substrate concentration of rhodopsin in our experiments may in fact be lower than that of β AR. However, this does not alter the fact that β AR is the preferred substrate for β ARK while rhodopsin is preferred by rhodopsin kinase.

The striking light dependence of rhodopsin phosphorylation by β ARK is demonstrated further in Fig. 3. At the 2-h time point a 60-fold increase in phosphorylation was observed in the presence of light. The low stoichiometry (~0.07 mol per mol) probably reflects the relatively low amount of β ARK used in these experiments. To compare the sites phosphorylated by rhodopsin kinase and β ARK, HPLC tryptic peptide mapping of phosphorylated rhodopsin was performed. The peptide maps appeared virtually identical, with only a single major peptide. Phosphoamino-acid analysis of this peptide revealed predominantly phosphoserine together with a small amount of phosphothreonine, for both kinases (data not shown).

Rhodopsin kinase phosphorylates rhodopsin at as many as nine different sites¹³, with seven of these residues (serines and threonines) being clustered in the carboxy-terminal 15 amino acids¹⁴. The sequence of the β -adrenergic receptor, deduced recently from the cloned gene, indicates that a similar serine- and threonine-rich region exists in its carboxy terminus³. The actual phosphorylation sites on the β AR have yet to be

Fig. 3 Time course of rhodopsin phosphorylation by the β -adrenergic receptor kinase. Urea-treated ROS (250 pmol of rhodopsin) were incubated at 30 °C with CM Fractogel-purified β ARK (0.3 μ g protein) in the dark (○) or under continuous illumination with white light (●) for the times indicated. The incubations also contained 20 mM Tris-HCl pH 7.5, 20 mM NaCl, 2 mM EDTA, 5 mM $MgCl_2$ and 0.05 mM [γ - ^{32}P]ATP (1,600 c.p.m. per pmol). Reactions were stopped by adding 50 μ l of SDS buffer before electrophoresis on a 10% polyacrylamide gel. After autoradiography the rhodopsin bands were excised and counted in a Packard scintillation counter. The results shown are representative of three experiments.

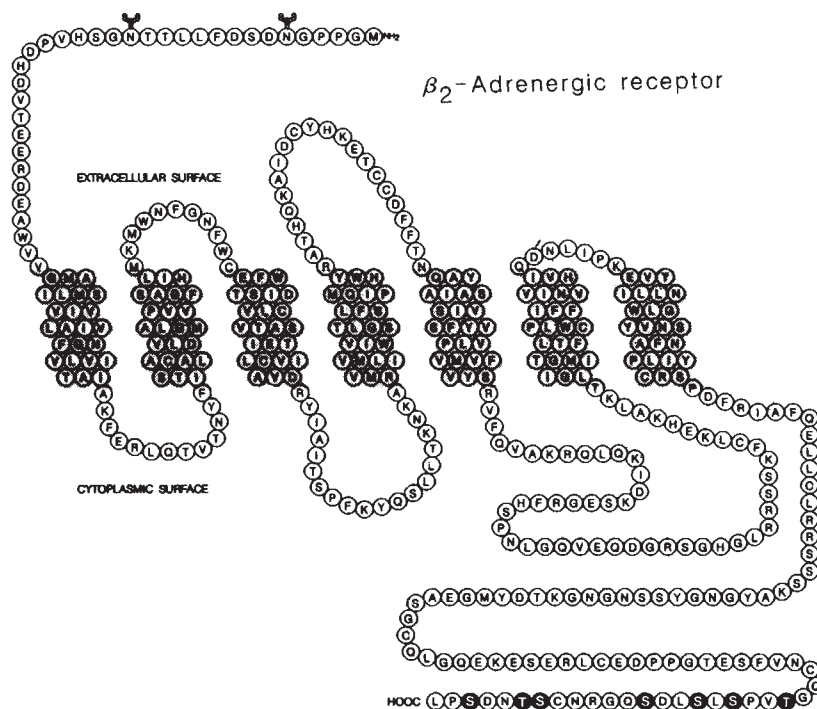
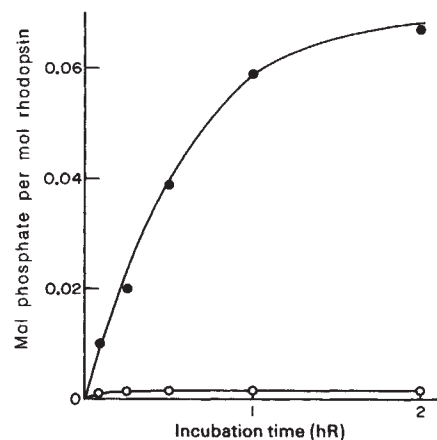
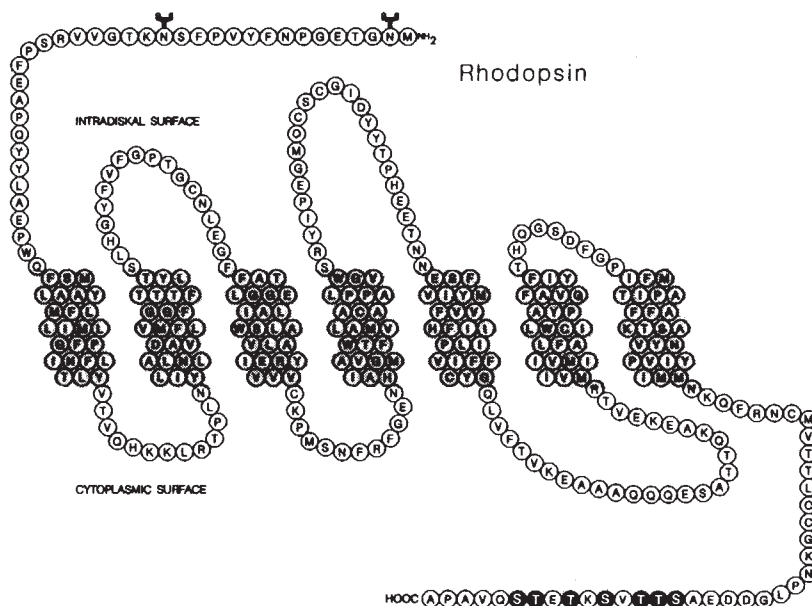


Fig. 4 Structure of the β -adrenergic receptor and rhodopsin polypeptide chains as they may be organized within the membrane. The seven known rhodopsin kinase phosphorylation sites on rhodopsin are represented by solid circles, as is a similar serine- and threonine-rich region near the carboxy terminus of β AR. Amino-acid sequences of the two proteins are taken from refs 3 and 20.



determined, however, the carboxy-terminal domain seems a likely region. Figure 4 compares the structures of the β AR and rhodopsin polypeptide chains as they may be organized within the membrane. The seven known phosphorylation sites on rhodopsin and seven possible sites of phosphorylation on the β AR are shown as solid circles.

We have found that β ARK, an enzyme which specifically phosphorylates only the agonist-occupied form of the β -adrenergic receptor, can also phosphorylate the same region on rhodopsin as does rhodopsin kinase, in a light-dependent manner. This result suggests that in addition to the striking homologies in the structure and function of signal transduction components which exist between the two systems, their biochemical mechanisms of regulation are also analogous. Phosphorylation of bleached rhodopsin by rhodopsin kinase appears to lead to the attenuation of its interaction with transducin¹⁵. Phosphorylation of the β -adrenergic receptor by β ARK in response to agonist occupancy of the receptor also appears to lead to an uncoupling of the receptor from the stimulatory guanine nucleotide regulatory protein, and this in turn is associated with attenuation of adenylate cyclase responsiveness (J.L.B., F.M. Jr, M.G.C. and R.J.L., manuscript in preparation).

Because phosphorylation of the receptor appears to be important for physiological adaptation (desensitization) of the responses associated with both the β -adrenergic receptor and the light receptor rhodopsin, phosphorylation may also represent an important control mechanism for other hormone-receptor systems. Recently, we have shown that desensitization of the α_1 -adrenergic receptor, a receptor coupled to the phosphatidylinositol- Ca^{2+} signal transduction pathway, is also

associated with its phosphorylation¹⁶. Moreover, desensitization or tachyphylaxis is a phenomenon which is common to many physiologically responsive systems. Thus, phosphorylation of the receptor component of hormone- and drug-responsive systems by a family of specific receptor kinases may represent a major mechanism by which cellular responsiveness to a variety of signals is regulated.

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Stimulation of haematopoiesis in primates by continuous infusion of recombinant human GM-CSF

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Certain proteins are known to play an important part in the proliferation, differentiation and functional activation of haematopoietic progenitor cells *in vitro*^{1,2}. These proteins include erythropoietin and various colony-stimulating factors (CSFs), one of which is granulocyte-macrophage colony-stimulating factor (GM-CSF). Recently, both murine^{3,4} and human GM-CSF⁵⁻⁷ have been purified to homogeneity and complementary DNAs encoding them have been cloned. Although the *in vitro* activity of recombinant human GM-CSF has been investigated intensively (refs 5-15; see ref. 8 for a review), little is known about the functional activity of this protein *in vivo*. There is strong evidence that colony-stimulating activities produced by various human and murine tumour tissues and cell lines can stimulate granulopoiesis in mice¹⁶⁻²⁶, as can human urinary extracts^{27,28}. A partially purified preparation of human urinary colony-stimulating factor, however, proved only marginally effective in stimulating granulopoiesis in humans²⁹. All these studies suffer from the lack of a homogeneous preparation of colony-stimulating factor. It has recently been shown that recombinant murine multi-CSF or interleukin-3 can stimulate haematopoiesis in mice *in vivo*^{30,31}. Large-scale produc-

tion of recombinant human GM-CSF now permits us to examine its effects *in vivo* using a primate model. We find that the continuous infusion of GM-CSF in healthy monkeys rapidly elicits a dramatic leukocytosis and a substantial reticulocytosis. A similar effect has been observed in one pancytopenic, immunodeficient rhesus macaque. These results suggest that GM-CSF could prove useful in several clinical situations.

To analyse the biological activities of recombinant human GM-CSF on simian progenitor cells, adherence-depleted simian bone marrow mononuclear cells were plated in 0.9% methylcellulose cultures at a cell concentration of 2.5×10^4 cells per ml. In this culture system, picomolar concentrations of purified human recombinant GM-CSF stimulated the terminal differentiation of simian granulocyte/macrophage progenitors (CFU-GM). Eosinophil colonies were not specifically counted (Fig. 1). These results support the earlier observation that conditioned media from the hairy-cell leukaemia cell line Mo can stimulate monkey granulocyte-monocyte colony formation³². In the presence of erythropoietin, the hormone also induced the proliferation and differentiation of early erythroid (BFU-E) and multipotential progenitors (CFU-GEM) (Fig. 1). These data confirm previous results obtained with human progenitors which show that GM-CSF is a multi-lineage haematopoietin¹¹⁻¹⁵. GM-CSF derived from *Escherichia coli* was as effective in stimulating colony formation as was the glycosylated protein derived from mammalian cells, indicating that carbohydrate modification has little effect on the *in vitro* activity of the haematopoietin (results not shown).

We next analysed the ability of GM-CSF to stimulate the proliferation of enriched populations of simian progenitor cells when plated at low cell densities (data not shown). To determine whether recombinant human GM-CSF acts directly on simian haematopoietic progenitor cells or indirectly through accessory cells, an additional panning step was performed on the adherence-depleted simian bone marrow mononuclear cells so as to remove T cells, B cells, monocytes, natural killer cells and granulocytes. This depletion did not affect the ability of GM-

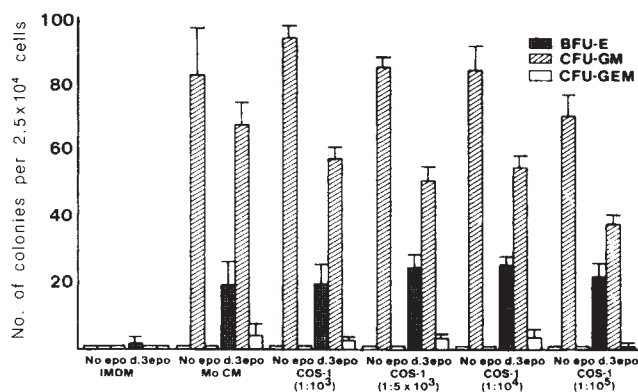


Fig. 1 Biological activity of HPLC-purified recombinant human GM-CSF derived from COS-1 cells on adherent-cell-depleted bone marrow obtained from a cynomolgous macaque (*Macaca fascicularis*) epo, erythropoietin. Bars show the mean ($\pm$ s.d.) of the colonies in duplicate plates.

Methods. GM-CSF expressed in COS-1 grown in serum-free medium was purified as described previously^{6,7}. The GM-CSF expressed in CHO cells grown in serum-free medium was also purified as described elsewhere^{6,7}. Heparinized bone marrow was aspirated from the femur of anaesthetized primates, resuspended 1:1 (v:v) in Iscove's modified Dulbecco's medium (IMDM) supplemented with 20% fetal calf serum (FCS) and the mononuclear cell layer was collected after density centrifugation at 20°C using a Ficoll-Paque (Pharmacia) gradient (specific gravity 1.077). The mononuclear cells were washed three times with IMDM + 20% FCS, resuspended at a concentration of 5×10^6 cells ml^{-1} , and depleted of adherent cells by incubating them for 3–4 h on 100 $\times$ 15-mm Lux tissue culture dishes (Miles Laboratories) pretreated with FCS. The non-adherent cells were gently removed and washed twice in IMDM + 20% FCS and the adherence depletion step was then repeated overnight. The non-adherent cells were collected, washed twice in IMDM, then resuspended at 2.5×10^4 cells ml^{-1} in 0.9% methylcellulose in IMDM with 30% FCS, 0.9% deionized bovine serum albumin (Sigma fraction V), and 10^{-4} M β -mercaptoethanol⁴³. Mo cell-conditioned serum-free medium was added to certain plates as 5% v/v. Highly purified GM-CSF expressed in COS-1 cells was diluted with IMDM so that the protein was at a concentration of $50 \mu\text{g ml}^{-1}$. This solution was then diluted still further to final dilutions of $1:10^3$ – $1:10^6$ in the methylcellulose cultures. One unit of erythropoietin (Terry Fox Laboratories, Vancouver) was added dropwise in each of the cultures that required the hormone 3 days after the initiation of incubation. The addition of erythropoietin was delayed to minimize or eliminate burst-promoting activity (BPA)-independent erythroid colony formation^{12–15}.

CSF to stimulate the formation of any of the different types of colonies when cultured at a low cell density ($1,000$ cells ml^{-1}). Although these experiments are not definitive, the results are consistent with other studies from our laboratory showing that the pluripoiectin activity of Mo cell-conditioned medium acts directly on human progenitors³³ and that antibodies directed against GM-CSF completely neutralize the pluripoiectin activity of Mo-conditioned medium (C. Sieff, personal communication).

To assess the importance of GM-CSF in regulating haematopoiesis *in vivo*, we examined the effects of continuous intravenous (i.v.) infusion of the protein on the levels of circulating blood cells in primates. We first analysed the rate of clearance of metabolically labelled haematopoietin from the circulation. The ^{35}S -labelled GM-CSF ($0.3 \mu\text{Ci } \mu\text{g}^{-1}$) was diluted with purified unlabelled GM-CSF ($100 \mu\text{g}$) and the mixture rapidly injected i.v. into a macaque. The clearance of acid-precipitable GM-CSF from the circulation (Fig. 2) followed the multiphasic kinetics typical of many glycoproteins³⁴. Initially, the level of radioactivity in the plasma decreased with an apparent half-life

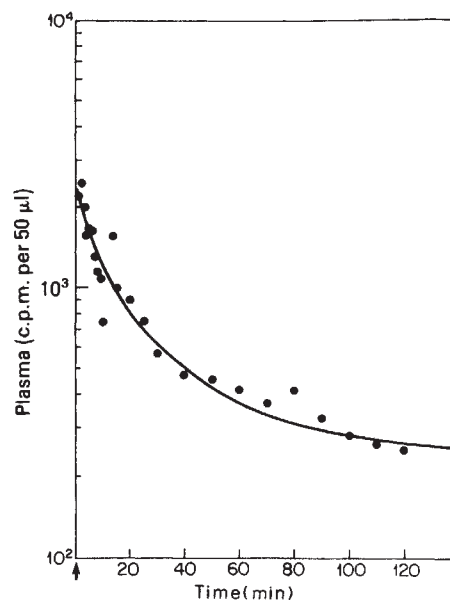


Fig. 2 Circulating plasma clearance of ^{35}S -methionine-labelled CHO-derived recombinant human GM-CSF. The initial, more rapid disappearance of radioactivity from the plasma is called the α phase, while the more prolonged disappearance is the β phase. The phases have a half-life of 7 min and 80–90 min, respectively. **Methods.** ^{35}S -radiolabelled GM-CSF was prepared from CHO cells expressing human GM-CSF by labelling 70% confluent cells with 5 mCi ^{35}S -L-methionine (NEN) in serum-free, methionine-free RPMI (Hazleton Research Laboratories, Denver, Pennsylvania). Supernatants were collected sequentially at 4 and 8 h. The GM-CSF was purified as described above to radiochemical homogeneity and had a specific activity of $0.3 \mu\text{Ci per } \mu\text{g}$ of protein. Approximately $25 \mu\text{g}$ of GM-CSF metabolically labelled with ^{35}S -methionine, together with $\sim 100 \mu\text{g}$ of non-radioactive GM-CSF, was inoculated as a single bolus into a cynomolgous macaque. Heparinized blood samples were collected at multiple time points. Aliquots ($50 \mu\text{l}$) of plasma were added to 1 ml of 5% cold trichloroacetic acid (TCA) and held on ice for 10 min. The samples were filtered through glass-fibre filters, washed with cold TCA, rinsed with cold methanol, dried and counted in anhydrous scintillation fluid (NEN) using a Beckman LS7800 scintillation counter.

of ~ 7 min. This initial decay (the α phase) of the protein is probably due largely to the distribution throughout the body fluid of the animal. A second decay component (the β phase) has an apparent half-life of 80–90 min and presumably represents the clearance of the protein by the animal's major organs. Even after 2 h, 10% of the GM-CSF remained in the circulation. Using the apparent half-life of the α phase, we estimated that continuous i.v. infusion at a rate of 50 U per min per kg would maintain the circulating levels of the hormone at a 5–10-fold higher concentration than required to stimulate optimal colony formation *in vitro*.

GM-CSF ($1\text{--}2 \times 10^7$ U per mg) was infused continuously at that rate through indwelling venous catheters into seven macaques for varying time intervals and blood samples were drawn daily for complete blood count analysis. Figure 3 shows clearly that the i.v. infusion of GM-CSF (Fig. 3b) dramatically increases the leukocyte count, which reaches a level seven times above the preinfusion average, whereas infusion of a control solution (Fig. 3a) has negligible effects on blood cell counts. Analysis of a bone marrow aspirate drawn from the monkey's femur on day 7 revealed that the marrow had become hypercellular during the infusion. This rise in leukocyte count was observed within 1–3 days of the start of infusion and was maintained by continued administration of GM-CSF.

Typically, the absolute numbers of neutrophils, band neutrophils, monocytes and lymphocytes all began to increase together. The effects of GM-CSF on circulating neutrophil, monocyte and eosinophil levels were consistent with the demonstrated *in vitro* activities of GM-CSF. This initial rise was usually followed by a 30-fold increase in the eosinophil level. After the infusion was stopped, the haematological profiles returned to normal. Usually, a modest stimulation in the levels of circulating reticulocytes could be observed during the infusion of GM-CSF (Fig. 3d) compared with the control infusion (Fig. 3c). However, repeated phlebotomy alone had a mild stimulatory effect on erythropoiesis during the control phase of the study, complicating the interpretation of the effect of GM-CSF treatment on this parameter. Results similar to those shown in Fig. 3 have been obtained with all seven animals tested thus far.

The leukocytosis elicited by GM-CSF infusion could be maintained for extended time periods by continued administration of the protein. In one experiment, a macaque infused for 28 days with GM-CSF had leukocyte counts of 30,000–90,000 throughout the infusion (data not shown) without apparent adverse effects. Although the initial leukocytosis obtained during the infusion may result from increased mobilization of mature granulocytes from the marrow, the maintenance of these high levels of circulating blood cells for long periods of time plus the observed marrow hypercellularity suggest that GM-CSF also has a direct effect on haematopoiesis. The maintenance of these high blood cell levels is absolutely dependent on continued administration of GM-CSF because the leukocytosis always resolved on termination of the infusion, usually within several days. In all cases in which we have treated normal animals, the complete haematological profile has always returned to normal.

To test the potential of GM-CSF in the treatment of cytopenias, we administered the protein to a severely debilitated, pancytopenic rhesus monkey (*Macaca mulatta*) that had been naturally infected with a simian type D retrovirus. The animal received ~500 U per min per kg of pyrogen-free recombinant human GM-CSF as a continuous subcutaneous (s.c.) infusion over 7 days. After an initial lag of 3 days, the monkey's leukocyte count began to increase significantly (Fig. 4a), rising from an initial level of 1,600 cells μl^{-1} (98% lymphocytes, 2% monocytes) to 23,000 7 days after the start of the infusion (52% neutrophils, 2% band neutrophils, 42% lymphocytes, 4% monocytes). The leukocyte count peaked at 43,700 on day 9 (2 days after the end of GM-CSF administration) and then began to decline, returning to 1,900 cells ml^{-1} by day 18. At the same dose, a healthy rhesus monkey demonstrated a similar response to the s.c. infusion of GM-CSF (Fig. 4b), its initial white blood cell count of 6,500 (31% neutrophils, 60% lymphocytes, 6% monocytes, 2% eosinophils, 1% basophils) rising to a maximum level of 64,300 (76% neutrophils, 4% band neutrophils, 7% lymphocytes, 4% monocytes, 8% eosinophils) 9 days after the start of the infusion. On termination of the infusion, the monkey's white blood cell count similarly returned to its initial level.

In the case of the severely pancytopenic monkey, we also observed a dramatic reticulocytosis elicited on infusion with GM-CSF (Fig. 4c). Biologically active erythropoietin was measured in both rhesus monkeys by a ^3H -thymidine uptake assay using spleen cells from phenylhydrazine-injected mice³⁵. The pancytopenic monkey was found to have higher levels of erythropoietin (320 mU ml^{-1}) than the normal animal (<50 mU ml^{-1} ; the assay used was sensitive to ~50 mU ml^{-1}), suggesting that the reticulocytosis observed in the pancytopenic animal may have resulted from a synergy between the infused GM-CSF and the high circulating level of erythropoietin.

Our results demonstrate that GM-CSF is a potent stimulator of haematopoiesis *in vivo*. Because the gene for GM-CSF is highly expressed in activated T cells as well as other cell types, the leukocytosis associated with certain infections may result in part from continued release of this and possibly other

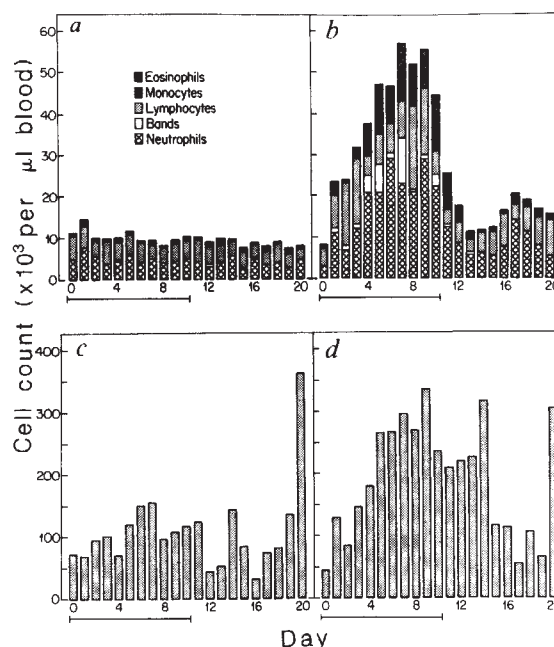
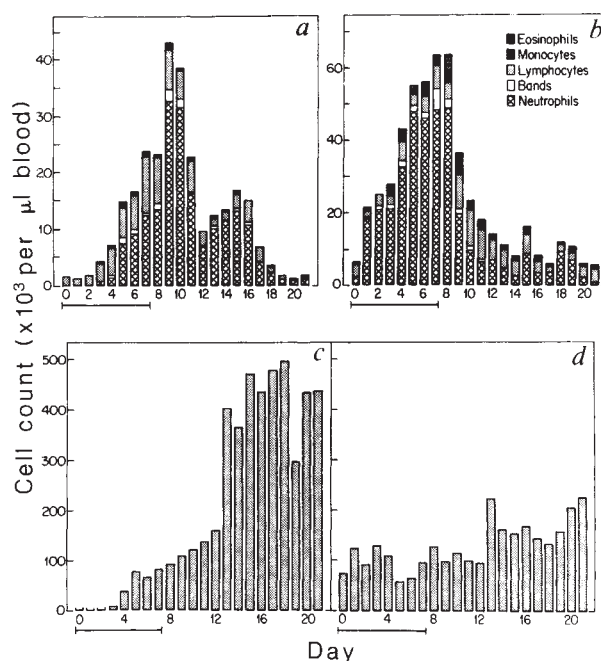


Fig. 3 Serial differential cell counts (a, b) and reticulocyte counts (c, d) of a healthy cynomolgous macaque (*M. fascicularis*) continuously infused with carrier alone (a, c) or recombinant human GM-CSF (b, d) for 10 days.

Methods. Primates were obtained from and housed at the New England Regional Primate Center. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council⁴⁴. GM-CSF expressed in CHO cells grown in serum-free medium was purified as described previously^{6,7}. The homogeneous protein from the final reverse-phase HPLC step was prepared for injection by repeated diafiltration against physiological saline (Baxter-Travenol, Deerfield, Illinois). Tween 80 (Rueger Chemical, Irvington, New Jersey) was added to a final concentration of 0.1% in the GM-CSF stock and diluted to 0.01% in 5% dextrose (Cutter Laboratories, Berkeley, California) immediately before infusion. Standard procedures were used to prepare and maintain samples pyrogen-free in the final formulated material. Endotoxin levels were determined by the *Limulus* amoebocyte lysate colorimetric assay (Whittaker Bioproducts, Walkersville, Maryland) and were undetectable (<0.1 endotoxin units ml^{-1}). The activity of the protein was determined by measuring chronic myelogenous leukaemia (CML) peripheral blood myeloblast incorporation of ^3H -thymidine in a CSF-dependent proliferation assay which has been described previously⁴⁵. CSF activity was measured relative to a standard preparation to which we had arbitrarily assigned an activity of 20,000 U ml^{-1} . In our assay system, the laboratory standard at a final concentration of 1 U ml^{-1} stimulates half of the maximal possible incorporation of ^3H -thymidine by CML peripheral blood myeloblasts. Our laboratory standard also has ~20,000 U ml^{-1} when tested in either the KG-1 or human bone marrow colony-forming assays as described previously^{6,7}. The preparation of GM-CSF used had a specific activity of $1\text{--}2 \times 10^7$ U per mg. The GM-CSF was infused at a rate of 50 U per min per kg using a continuous infusion pump (Ferring Laboratories, Ridge-wood, New Jersey) designed to deliver at a rate of 50–75 $\mu\text{l h}^{-1}$. Indwelling i.v. catheters were placed in either the iliac or jugular veins. Blood samples were taken after anaesthetizing the animal with ketamine hydrochloride (Bristol Laboratories, Syracuse, New York) and submitted in EDTA for serial blood counts.

Fig. 4 Serial differential cell counts (*a, b*) and reticulocyte (*c, d*) counts of a pancytopenic rhesus monkey (*a, c*) and a healthy rhesus monkey (*b, d*) continuously infused with recombinant human GM-CSF for 7 days.

Methods. GM-CSF was administered s.c. for 1 week using Model 2ML1 Alzet osmotic pumps (Alza, Palo Alto, California) which were surgically implanted. The pancytopenic animal had profuse diarrhoea and was later determined to have been naturally infected with a type D retrovirus by methods previously described⁴⁶.



haematopoietins. We are now designing experiments to test this hypothesis. In addition, we are analysing the functional properties of the circulating cells elicited by GM-CSF treatment to assess its role in the activation of mature leukocytes.

The demonstration that GM-CSF is a potent stimulator of haematopoiesis in normal primates provides the opportunity to develop models for testing potential clinical uses of the protein. For example, we have already shown here that virally induced cytopenias, which are a common sequelae of type D infections in simian primates³⁶⁻³⁸, may respond to GM-CSF therapy. Moreover, GM-CSF therapy may prove useful in the treatment of other virally induced cytopenias³⁹, such as is often associated with human AIDS (acquired immune deficiency syndrome)^{40,41}. The present results also suggest that GM-CSF could prove valuable in patients undergoing bone marrow transplantation or cancer therapy. By shortening and possibly eliminating the leukopenia associated with these treatments, the risk of secondary infections would be reduced. One such approach has

already been discussed⁴². The availability of the recombinant human protein should enable us to address these questions.

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No functional γ -chain transcripts detected in an alloreactive cytotoxic T-cell clone

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Three groups of genes that undergo rearrangements during T-cell maturation have been isolated from T cells¹⁻¹³. Two of them encode the α - and β -subunits of the T-cell antigen receptor and are shared between antigen-specific, major histocompatibility (MHC) class I-restricted cytotoxic T cells and antigen-specific, MHC class II-restricted helper T cells^{1-10,14}. The third group of genes, called γ , is preferentially transcribed in cytotoxic T cells^{12,15}. This led to the hypothesis that the unidentified γ -gene products could be part of a putative T-cell receptor responsible for MHC class I recognition^{16,17}. We report here on the isolation of three different types of γ -gene transcripts of an alloreactive cytotoxic T-cell clone (3F9). Two are derived from two rearrangements that have occurred at the same locus (V_γ 10.8A to J_γ 10.5 and transcribed with C_γ 10.5), while the third involves a new V_γ -gene segment that is joined to J_γ 13.4 and transcribed with C_γ 13.4. All these rearrangements are abortive and lead to the formation of non-functional γ -chain genes because the proper translational reading frame is not maintained. Because the second copy of the C_γ 13.4 gene segment is deleted and as C_γ 7.5 is considered to be a pseudogene and has not undergone any rearrangements in 3F9, we conclude that the alloreactive cytotoxic T-cell clone 3F9 does not contain a functional transcript of a known γ -chain gene.

3F9 is an alloreactive cytotoxic T-cell clone of BALB/c ($H-2^d$) origin and is specific for the D^b allele of the murine MHC locus¹⁸. A complementary DNA library of 3F9 in λ gt-11 was constructed¹⁴ and screened with a ³²P-labelled oligonucleotide for γ -sequence-bearing clones. Nine γ -positive cDNA clones were isolated, analysed by restriction mapping and sequenced. Three distinct groups of cDNA clones were identified. Figure 1 shows the restriction map of a representative clone from each group (3F9- γ 4, - γ 6 and - γ 7) and the respective sequencing strategy, while Fig. 2 shows the corresponding nucleotide sequence. 3F9- γ 4 and - γ 7 are transcripts derived from the same gene segments (V_γ 10.8A, J_γ 10.5 and C_γ 10.5) but are different at the variable-joining (V - J) junction (Fig. 2). They therefore represent two rearrangement events involving both copies of the same gene on both chromosomes.

Although 3F9- γ 4 and - γ 7 use the same V_γ 10.8A gene segment, they differ by one nucleotide in the variable region (position 216, Fig. 2). If this difference is not due to a cloning artefact (which we cannot rule out), it might be a somatic mutation, since the T-cell clone 3F9 is derived from a homozygous inbred mouse strain (BALB/c). 3F9- γ 6 is a representative of the third group. Although it is not a full-length cDNA clone, it carries nucleotide sequences similar to the already known V_γ genes. The presumptive V_γ region of 3F9- γ 6 is ~70% homologous with the three known variable regions V_γ 10.8A and B and V_γ 5.7 (ref. 13). We therefore conclude that 3F9- γ 6 carries part of a new V_γ gene which has not been identified previously at the genomic level. The J_γ region of 3F9- γ 6 is identical to the genomic J_γ 13.4 gene segment and the constant region is different but highly homologous to the genomic C_γ 10.5 and C_γ 7.5 (ref. 13). For these reasons we believe that the C_γ gene expressed in 3F9- γ 6 is derived from the genomic C_γ 13.4. Figure 2 also compares the nucleotide sequences of the C_γ regions of 3F9- γ 4 (corresponding to C_γ 10.5), 3F9- γ 6 (presumably C_γ 13.4) and the genomic C_γ 7.5 (ref. 13). Both C_γ 7.5 and C_γ 6 gene segments have a 15-nucleotide insertion within the coding region at posi-

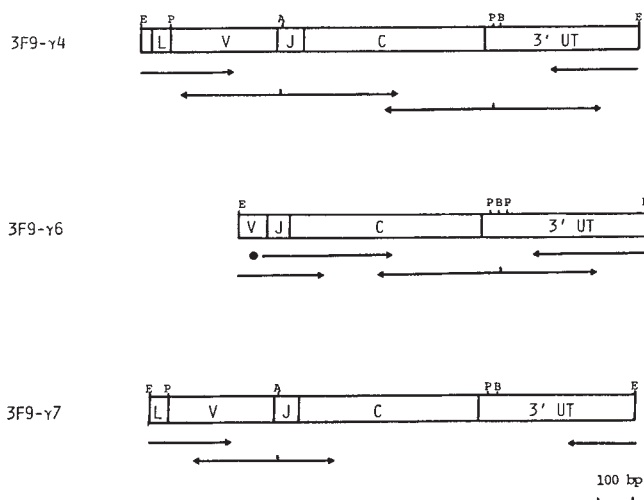


Fig. 1 Restriction map and sequence strategy of the longest inserts from the three different groups of 3F9- γ cDNA clones. Restriction enzyme sites are indicated by small letters: E, *Eco*RI; P, *Pvu*II; B, *Ban*I; A, *Ava*I. Part of the sequence of 3F9- γ 6 was derived from a shorter clone of the same group (indicated by $\bullet \rightarrow$) sharing the same 3' end of the $V_{\gamma 6}$ gene segment and the same V - J junction.

tion 776; this insert conserves the original reading frame. C_γ 6 differs from C_γ 10.5 and C_γ 7.5 by 53 and 37 nucleotides, respectively. Between the 3' end of V_γ 10.8A and the 5' end of J_γ 10.5 in 3F9- γ 4, the sequence CCCC can be found. These nucleotides are not present in the corresponding germline gene segments. In 3F9- γ 7 six nucleotides at the 3' end of the V_γ 10.8A gene segment have been deleted and the dinucleotide AT precedes the J_γ 10.5 gene segment. These sequences could represent a portion of an as yet unidentified D (diversity) gene segment or the result of a terminal deoxynucleotidyltransferase activity during V - J rearrangement. All these rearrangements, however, produce non-functional RNA transcripts, since the V_γ - J_γ joining did not result in a proper translational reading frame. Stop codons are present in the J segments of 3F9- γ 4 and - γ 6 and at the V - J junction in 3F9- γ 7 (Fig. 2).

To evaluate whether the second copy of the C_γ 13.4 gene segment located on the homologous chromosome could have been rearranged, we analysed the γ -chain gene rearrangements at the genomic level by Southern blot analysis. Considering the length of the restriction fragments bearing the V_γ and C_γ gene segments, it can be predicted that the rearrangement of V_γ 10.8A to J_γ 10.5 creates a new band of ~16 kilobases (kb)¹³. The Southern blot (Fig. 3) shows that both chromosomes at the V_γ 10.8A and J_γ 10.5 loci have undergone rearrangements in 3F9 (as expected from the analysis of 3F9- γ 4 and - γ 7) since a band at 16 kb can be detected and the germline bands at 10.5 and 10.8 kb are no longer seen. The germline band at 13.4 kb, containing the C_γ 13.4 locus, also disappears and a new one at ~20 kb emerges. Hybridization with a ³²P-labelled 3F9- γ 4 probe (bearing V_γ 10.8A and C_γ 10.5) or with a 3F9- γ 6 probe (bearing part of the new V_γ gene and C_γ 13.4) shows that this band is of much lower intensity and probably contains only one rearranged copy of the C_γ 13.4 segment (Fig. 3a). The 16-kb band containing two rearranged C_γ 10.5 gene segments and the 7.5-kb band with the two non-rearranged C_γ 7.5 gene segments are clearly more intense than the 20-kb band bearing the rearranged C_γ 13.4 locus (this analysis has been done five times with identical results). This finding and the fact that no additional rearranged bands can be detected are strong circumstantial evidence that the second copy of the C_γ 13.4 gene segment on the homologous chromosome has been deleted. This conclusion is also strengthened by the cDNA screening results. From the nine

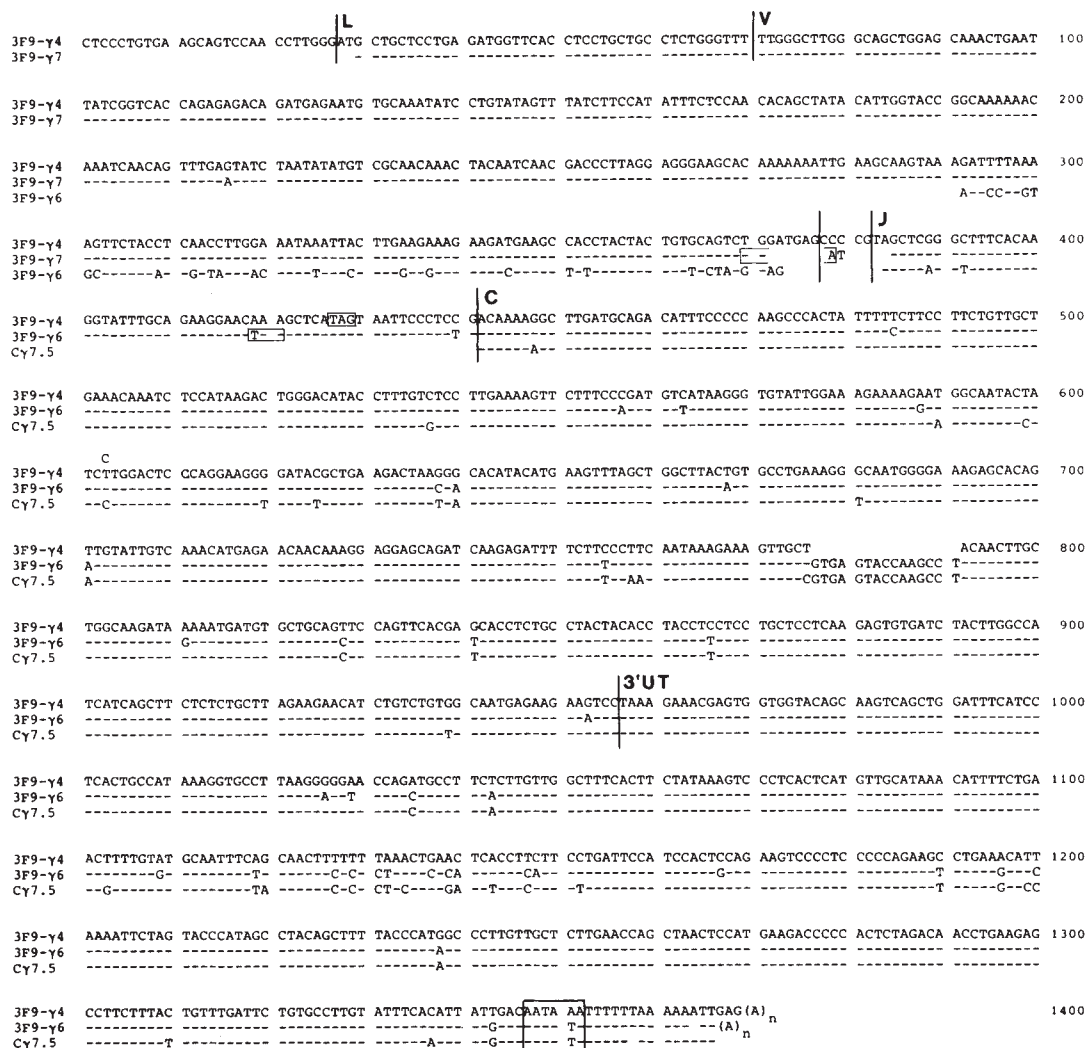


Fig. 2 Nucleotide sequence of 3F9- $\gamma 4$, $\gamma 6$ and $\gamma 7$ cDNA clones. The sequence of the genomic $C_{\gamma}7.5$ gene segment¹³ is also indicated. The synthetic oligonucleotide used for screening was complementary to the C_{γ} region from position 459 to 482 and was synthesized using an Applied Biosystems 380A DNA synthesizer. The construction of the λ gt11-3F9 cDNA library has been described elsewhere¹⁴ and the nucleotide sequence analysis was performed according to the technique of Maxam and Gilbert²². The stop codons and the polyadenylation signals of the different C_{γ} -gene segments are boxed. The 3F9- $\gamma 4$ cDNA clone and the genomic $C_{\gamma}10.5$ gene segment show a difference at position 603 (T $\rightarrow$ C).

γ -gene transcripts isolated out of 10^5 recombinant phages, six are derived from the rearranged $C_{\gamma}10.5$ locus (3F9- $\gamma 4$ and - $\gamma 7$ types) and the other three, which have all been sequenced, are identical and are exclusively derived from one type of rearrangement event at the $C_{\gamma}13.4$ locus (3F9- $\gamma 6$ type).

The $\gamma 6$ probe reveals an additional faint band at 5.0 kb in the germline DNA; this probably represents a genomic V_γ gene segment that cross-hybridizes to the 3F9- $V\gamma 6$ gene (Fig. 3a, lane 3). The weak signal intensity is probably due to the fact that the 3F9- $\gamma 6$ probe contains only one-third of the complete V_γ gene segment.

Another observation concerns the pseudogene *C_γ7.5* (ref. 13). This gene segment contains an altered splice donor site and carries an unusual polyadenylation signal, AATATA instead of AATAAA. Interestingly, the sequence of *C_γ13.4* in 3F9-γ6 shows exactly the same altered polyadenylation signal but, despite this, it is polyadenylated correctly. Therefore, *C_γ7.5* should be a pseudogene as a result of its altered splice donor site and not because of its different polyadenylation signal. Also, *C_γ7.5* and *V_γ5.7* are absent from the genome of B10 mice, and in 3F9 (BALB/c) they are neither rearranged nor transcribed (Fig. 3b).

The absence in B10 could imply that there is no ‘positive’ selection pressure on this gene, further indicating that it could represent a non-functional gene.

Our study shows that multiple rearrangement events can occur at different γ loci and that the $V\gamma 10.8A$ gene segment is always expressed with the $J\gamma 10.5$ and $C\gamma 10.5$ genes as described previously^{12,15}. From these findings and from the fact that each $C\gamma$ gene segment carries its own $J\gamma$ gene segment, we believe that the γ -gene family is organized similarly to the immunoglobulin λ -light-chain gene family. In the λ -chain family, two different $V\lambda$ regions can only rearrange to a limited number of J -gene segments which are associated with their respective $C\lambda$ genes¹⁹⁻²¹.

The finding that both the $C_{\gamma}7.5$ and the $V_{\gamma}5.7$ gene segments are missing in the C57BL/10 mouse strain indicates that these genes could be associated as a separate set of V_{γ} , J_{γ} and C_{γ} gene segments. Since a functional γ -chain transcript could not be detected in the alloreactive cytotoxic T-cell clone 3F9, we conclude that γ -chains are not necessary for the killing mechanism, at least for alloreactive cytotoxic T cells. Furthermore, if alloreactive and antigen-specific, MHC-restricted cytotoxic T

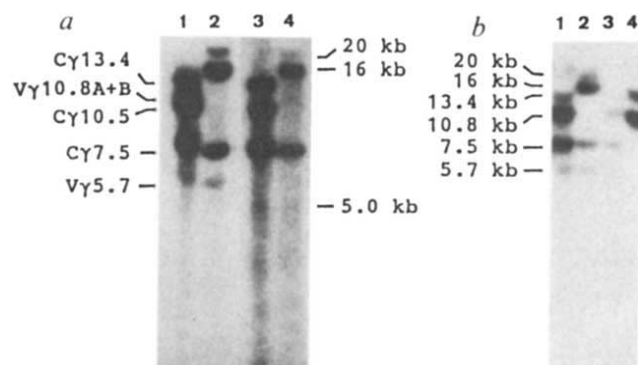


Fig. 3 Southern blot analysis of genomic and 3F9 DNA. *a*, Lanes 1 and 2 probed with ^{32}P -labelled 3F9- $\gamma 4$, lanes 3 and 4 with 3F9- $\gamma 6$. BALB/c kidney (lanes 1, 3), 3F9 (lanes 2, 4). *b*, BALB/c kidney, 3F9, C57BL/6 and C57BL/10 kidney DNA (lanes 1-4, respectively) probed with ^{32}P -labelled 3F9- $\gamma 4$. DNA was extracted from either kidney or 3F9 T cells, digested with *EcoRI*, electrophoresed through 0.5% (*a*) or 0.7% (*b*) agarose gels, treated with 0.25 M HCl and transferred to nitrocellulose²³. The filters were hybridized to ^{32}P -labelled probes, washed in $0.3 \times \text{SSPE}$, 0.1% SDS at 65 °C and autoradiographed. Each Southern blot analysis was performed five times.

cells use the same recognition mechanism via the $\alpha\beta$ T-cell receptor, then the γ -chains are not relevant for recognition. If, however, the still elusive γ -chains are somehow involved in MHC class I recognition in antigen-specific, MHC-restricted cytotoxic T cells, then the recognition mechanism of alloreactive versus antigen-specific, MHC-restricted cytotoxic T cells is different. This could imply that alloreactive cytotoxic T cells either do not need a second receptor for recognition and mediation of effector function or that the determinants functioning as 'restriction' elements of these T cells are recognized by some other unidentified molecules.

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Note added in proof: Iwamoto *et al.* (*J. exp. Med.*, in the press) have isolated a cDNA clone from a hapten-specific T cell bearing a known V_γ region associated with a new $J-C$ region which is similar to, but does not crosshybridize with, any known C_γ region genes. We have isolated similar γ -like transcripts from 3F9, but do not yet know if these clones contain an open reading frame.

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A functional γ gene formed from known γ -gene segments is not necessary for antigen-specific responses of murine cytotoxic T lymphocytes

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Structural similarities between surface immunoglobulins (s Ig) on B cells and antigen-specific receptors on T cells suggest that a T cell, like a B cell, should express only two immunoglobulin-like genes, one for each subunit of the disulphide-linked, heterodimeric, antigen-specific ($\alpha\beta$) T-cell receptor. However, cytotoxic T lymphocytes (T_c cells) and immature thymocytes also contain RNA transcripts of a third immunoglobulin-like gene, called γ (refs 1-4). A polypeptide corresponding to the γ gene has not yet been identified and the function of this gene remains an enigma. Judging from its nucleotide sequence, the rearranged γ gene is expected to encode an integral membrane polypeptide chain, and γ complementary DNAs from two cloned T_c cell lines have previously been found to have different sequences around the $V-J$ (variable region-joining region) junction^{1,2}, suggesting that, in these cells, the γ -gene product is a clonally diverse surface structure that may form part of an as yet unidentified, antigen-specific receptor. To analyse further the extent of diversity of the γ -gene product, we have determined the partial sequences of 11 γ cDNA clones from three other cloned T_c cell lines, and report here that the sequences are indeed clonally diverse, but in all instances they are out-of-phase in the region of the $V-J$ junction. This finding and the pattern of γ -gene rearrangements in these cell lines indicate that a polypeptide product of the previously reported γ gene, $V2J2-C2$, is not expressed in them and is, therefore, not necessary for the antigen-specific cytotoxic and proliferative responses of these mature T cells.

The cloned T_c cell lines examined (described in Table 1) were derived from BALB.B ($H-2^b$) and BALB.K ($H-2^k$) mice that had been injected several times ('hyperimmunized') with P815 cells (a DBA/2 ($H-2^d$) mastocytoma). The cell lines were specific for the class I histocompatibility antigen L^d or D^d and had the $L3T4^+$, $Lyt\ 2^+$ phenotype expected of T_c cells. cDNA libraries were prepared from these cell lines, γ -positive cDNA clones were identified, and partial nucleotide sequences were determined (see Fig. 1).

Hayday *et al.*⁵ previously described three V , and J and three C (constant) γ -gene segments and demonstrated that the J_γ and C_γ gene segments are paired as in the immunoglobulin λ gene family^{6,7}. To simplify discussion, these γ -gene segments are referred to here as follows (their previous designations, based on the size, in kilobases (kb), of the *EcoRI* fragment on which each is found, are given in parentheses): $V1$ (10.8B), $V2$ (10.8A), $V3$ (5.7), $J1-C1$ (13.4), $J2-C2$ (10.5) and $J3-C3$ (7.5).

Except for variations in the region of the $V-J$ junction, the sequences of the 11 cDNA clones were all identical to the previously described sequence of γ cDNA clones (pHDS 4/203)¹ from the T_c cell line 2C (also of BALB.B origin and specific for L^d)⁸. As shown in Fig. 1, in clone pHDS 4/203 from 2C cells, the insertion of an A-T dinucleotide between the $V2$ and $J2-C2$ gene segments established an in-frame join; the resulting sequence can encode a full-length γ polypeptide chain^{1,2}. However, for all the γ cDNA clones from the three cell lines listed in Table 1, the various sequences in the vicinity of the $V-J$ junction result in out-of-frame joins that are not expected to yield full-length γ chains. Thus, for γ cDNA from 3H-2 cells

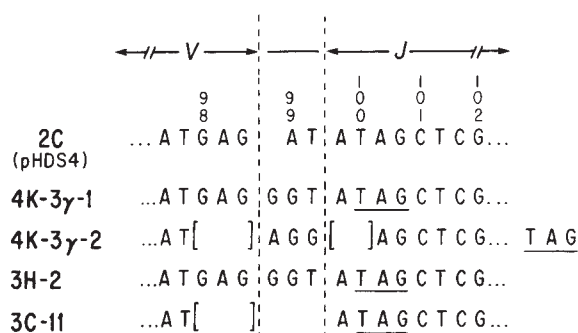


Fig. 1 Nucleotide sequences of γ cDNA clones in the region of the V-J junction. Nucleotides shown between the vertical broken lines are not encoded by known V_γ or J_γ gene segments⁵. Brackets indicate deletions. The codons are numbered according to the previously described γ clone pHDS4 from the T_c line 2C^{1,2}, and premature termination codons are underlined (the termination codon for 4K-3 γ -2 occurs ~45 nucleotides downstream).

Methods. cDNA libraries were prepared in λ gt10 essentially as described elsewhere¹⁵, and screened with a C_γ probe (pHDS4)¹. For every 100,000 plaques screened, 3-4 C_γ -hybridizing plaques and 60 C_β -hybridizing plaques were detected, on average. A total of 11 γ cDNA were sequenced by the method of Maxam and Gilbert¹⁶: 4 of the 4K-3 γ -1 type, 2 of the 4K-3 γ -2 type (both from 4K-3E), 4 of the 3H-2 type, and 1 of the 3C-11 type.

and for one of the γ cDNA clones from 4K-3E cells (4K-3 γ -1), an insert of three nucleotides (GGT) at the V-J junction results in premature chain termination. In another γ clone from 4K-3E cells (4K-3 γ -2), a different set of three nucleotides (AGG) is inserted at the V-J junction and both the last three nucleotides of the V2 gene segment (GAG) and the first two nucleotides of the J2 gene segment (AT) are deleted; the result is a termination codon 45 nucleotides downstream of the V-J junction. Finally, in the γ cDNA clones of 3C-11 cells, no nucleotides are inserted between the 3' end of V2 and the 5' end of J2 gene segments and there is a deletion of the last three nucleotides of V2; the result is a termination codon in the J region (Fig. 1).

The significance of the aberrant γ transcripts in the cell lines listed in Table 1 is apparent on inspection of their respective γ -gene rearrangements. As shown in the Southern blot hybridization patterns (Fig. 2), the 4K-3E cell line, like the previously described 2C cell line², has both the 16- and the 22-kb rearranged γ -positive fragments. In 2C cells the 16-kb fragment was shown to contain a rearranged γ gene corresponding to the assembly of V2J2-C2 gene segments⁵. As there are no differences between the 341 sequenced nucleotides of 4K-3 γ -1 (data not shown) and the V2J2-C2 γ gene of 2C (except at the region of the V-J junction), it is probable that this 4K-3E γ cDNA clone represents a V2J2-C2 γ gene on the 16-kb fragment. Indeed, there are probably two rearranged V2J2-C2 γ genes in the 4K-3E cell line (one from each chromosome), because these cells do not have the germline configuration of the J2-C2 γ -gene segments (Fig. 2, lane 5). It is thus of interest that a second γ cDNA clone (4K-3 γ -2) from 4K-3E cells also has an out-of-frame V-J join (Fig. 1). The rearrangements of some of the other known gene segments also could not encode a γ -chain in these cells: thus, the third C_γ gene segment (C3) is defective⁵, and a γ gene resulting from rearrangement of V_3 to J1-C1 or to J2-C2 would have been detected in Fig. 2 (see ref. 5). The 22 kb rearranged fragment in 4K-3 cells will be discussed below.

Analyses of two other cell lines, 3H-2 and 3C-11, also suggested that a polypeptide chain encoded by the V2J2-C2 γ gene^{2,5} need not be expressed in cytolytically active T_c cells. Southern blots revealed that each of these cell lines has a 16-kb,

Table 1 Cloned cytotoxic T cells

Clone	Strain of origin	Specificity	Lyt 2	L3T4
3C-11	BALB.B ($H-2^b$)	D ^d	+	-
3H-2	BALB.K ($H-2^k$)	D ^d	+	-
4K-3	BALB.K ($H-2^k$)	L ^d	+	-

BALB.B ($H-2^b$) or BALB.K ($H-2^k$) mice were injected intraperitoneally with $\sim 2.5 \times 10^7$ irradiated (3,000 rad) P815 cells (a DBA/2 ($H-2^d$) mastocytoma) at 3-week intervals, either three (clones 3C-11 and 3H-2) or four (clone 4K-3) times; 1-2 weeks after the final injection, alloreactive cloned T_c lines were derived from spleen cells as described elsewhere. Specificity in target cell lysis was determined by a standard 4-h ⁵¹Cr-release assay¹⁴ using as targets mouse L-cells transfected with D^d (cell line T4.8.3) or L^d (cell line T1.1.1) class I MHC antigens (provided by J. Seidman). At an effector/target cell (ratio of 5:1, the per cent ⁵¹Cr-release from the appropriately labelled target cell was 58%, 83% and 39% for lines 3C-11, 3H-2 and 4K-3, respectively. The L3T4⁺, Lyt 2⁺ surface phenotype was determined by fluorescence-activated cell sorting (System 50-H, Ortho Diagnostic) using rat monoclonal antibodies against Lyt 2 (536.7; given by F. Fitch) and L3T4 (GK1.5; given by K. Wall).

fragment with a rearranged γ gene (Fig. 2). Moreover, the blots showed that these cells also contain unrearranged germline C_γ gene segments (compare lanes 1-4 of Fig. 2; see ref. 5). Thus it seems that in both the 3H-2 and 3C-11 cell lines there is only a single rearranged V2J2-C2 γ gene in the γ -positive 16-kb fragment, and in both cell lines this gene is not functional. Thus, four γ cDNA clones from 3H-2 cells yielded the same sequence, which was identical to that of pHDS 4/203 of 2C cells except for the out-of-frame join in the region of the V-J junction (Fig. 1). For the 3C-11 cell line only a single γ cDNA clone was analysed and it also had an out-of-frame V-J join (Fig. 1). As these cell lines have been found (S. Latt, personal communication) to be predominantly diploid (80-85% of the cells in the 3C-11 clone and 65% of those in 4K-3L are diploid), these results suggest strongly that all of their rearranged V2J2-C2 γ genes have out-of-frame joins.

Taking all the above results together, all three of the cytolytically active T_c cell lines studied here appear to be incapable of producing a γ -chain that is encoded by the V2J2-C2 γ gene. Some additional V_γ gene segments, not cross-hybridizing with the V1, V2 and V3 γ -gene segments, have recently been identified (J. Heilig and S.T., manuscript submitted and ref. 18) and found, when rearranged, to be joined with the J1-C1 segment; hence they would have been detected by screening libraries with probes that hybridize with the C_γ 1 gene segment. Moreover, since both of the J2-C2 gene segments in 4K-3 cells are rearranged to V2 in the 16-kb fragment, it seems unlikely that the newly identified V_γ gene segments (or any additional ones) can be expressed in these cells, unless they are rearranged to as yet unidentified J-C γ -gene segments; if such gene segments exist, they do not cross-hybridize with the known J-C γ -gene segments. The arguments applied here to some cloned T_c cells also apply to certain T-helper (T_H) cells, which do not express the previously described γ -gene segments⁹. Of course, the conclusion that functional T_c and T_H cells need not express γ -chains must be considered provisional for both cell types, because it is possible that as yet unidentified V_γ and J_γ - C_γ gene segments, not cross-hybridizing with the known γ -gene segments, might encode γ -chains in these cells.

Could 4K-3 cells have in-frame γ transcript of a rearranged γ gene of the 22 kb fragment? This fragment has recently been shown to represent an incomplete *Eco*RI digest of a rearrangement in which the newly identified V_γ 4 gene segment is joined to the J1-C1 gene segment (J. Heilig and S.T., in preparation). Complete digestion yielded a somewhat smaller fragment not easily resolved from the 16 kb (V2J2-C2) fragment. Thus, complete digestion of the V J1-C1 γ gene may account for the

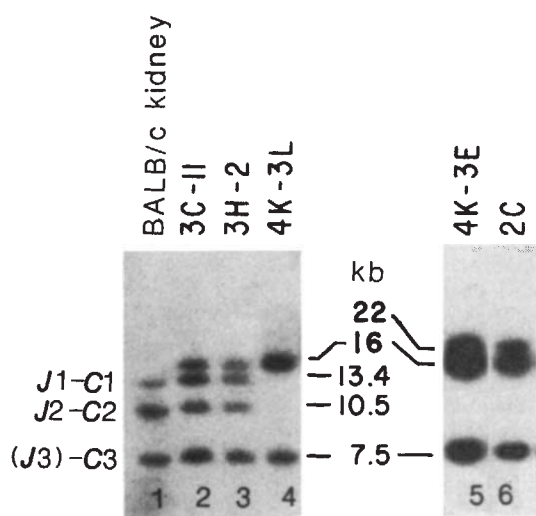


Fig. 2 Southern blot analysis of T_c DNA hybridized to a C_γ probe (pHDS4)¹. DNA from BALB/c kidney (lane 1) has germline $J1-C1$, $J2-C2$ and $(J3)-C3$ gene segments on fragments of 13.4, 10.5 and 7.5 kb, respectively. DNAs from T_c lines 3C-11 and 3H-2 (lanes 2 and 3) have germline (that is, unrearranged) 13.4-kb ($J1-C1$) and 10.5-kb ($J2-C2$) fragments. The Southern blot of DNA from the 4K-3 cells (4K-3E) was repeated from cells that had been in culture for about 6 months (4K-3L). The only difference was the presence of a 22 kb fragment in the DNA from 4K-3E cells. This fragment represents an incomplete digest of a $V4J1-C1$ γ gene rearrangement. The 4K-3L cells retained specificity for the L^d antigen and were virtually as active cytolytically as 4K-3E cells. DNAs (2–3 μ g of each) were digested with *Eco*RI, electrophoresed through 0.8% agarose gels and blotted onto nitrocellulose¹⁷. Blots were hybridized at 42 °C in 50% formamide and 5 $\times$ SSC and filters were washed at 65 °C in 0.2 $\times$ SSC.

absence of the 22 kb fragment in the Southern blot of 4K-3 cells that had been in continuous culture for about six months (designated 4K-3L cells, Fig. 2, lane 4). In any case, the $V\gamma 4$ gene segment does not seem to be transcribed in 4K-3 cells or in any of the other mature T cell lines analysed thus far (unpublished observations).

The relative abundance of γ transcripts in immature thymocytes has prompted the speculation that γ -containing, antigen-specific receptors of these cells may be involved in the intrathymic selection of self-MHC (major histocompatibility complex)-restricted T cells, that is, lymphocytes that can distinguish between self- and non-self-MHC^{3,4}. This distinction underlies the MHC-restriction of antigen recognition that is characteristic of T_c (and T_h) cells. There are no reasons to doubt that the three cell lines analysed here, although alloreactive (anti- L^d or D^d), are representative of typical MHC-restricted T_c .

cells. Whether they have ever expressed a functional γ transcript is thus a question of considerable interest. It seems unlikely that the $V2J2-C2$ γ gene could have been expressed at any time in 4K-3, 3H-2 or 3C-11 cells because in these cells all copies of this gene are out-of-phase. However it is possible that a functional γ gene could have been expressed in immature progenitors of the cloned 4K-3, 3C-11, and 3H-2 cell lines formed by rearrangement of one of the recently identified $V\gamma$ gene segments to $J1-C1$ (J. Heilig and S.T., manuscript in preparation and ref. 18).

Finally, we note that altogether in the four distinct γ cDNA clones analysed here, more than 500 V_γ and J_γ nucleotides were sequenced and, except for those at the $V-J$ junction, all are identical to those in the germline $V2$ and $J2$ gene segments⁵. The absence of nucleotide replacements is notable because the γ cDNA clones analysed were from cloned T_c cell lines that had been derived from hyperimmunized mice. In contrast, a previous analysis of immunoglobulin light-chains ($\lambda 1$) transcripts from hyperimmunized mice revealed that V-domain nucleotide substitutions in these chains occurred with an average frequency of ~ 0.01 (that is ~ 3 per 330 nucleotides; ref. 10 and K. Tamoto, E. B. R. T. Azuma and H.N.E., in preparation). If somatic mutations in the transcribed immunoglobulin-like genes in T cells from hyperimmunized mice matched in frequency those in immunoglobulin light chain ($\lambda 1$) genes in such mice, five nucleotide replacements would have been expected in the γ sequences determined here. As none were seen, our findings support growing evidence that somatic mutations are less frequent in T than in B cells^{11,12}. One objection which could be raised is that the absence of somatic mutations in a rearranged γ gene that is not expressed as a polypeptide chain is not relevant for the frequency of somatic mutations in α and β genes, which are expressed as polypeptide chains. However, somatic mutations in a rearranged out-of-phase κ gene have been described¹³. Moreover, it seems reasonable to expect that cells in which somatic mutations have occurred in immunoglobulin and immunoglobulin-like genes that are expressed as polypeptide chains may well also have mutations in rearranged immunoglobulin-like genes that are transcribed but not translated into protein. Indeed, the latter mutations should be particularly informative, because they are not subject to selection by antigen or other agents (for example anti-idiotypes).

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Note added in proof: Recently another $J\gamma C\gamma$ gene segment ($J4-C4$) which does not crosshybridize with the previously reported JC segments has been described by Iwamoto *et al.* (*J. exp. Med.*, in the press).

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Corticotropin releasing factor induction of leukocyte-derived immunoreactive ACTH and endorphins

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Human peripheral leukocytes^{1,2} infected by virus or treated with endotoxin will, like unstimulated mouse spleen macrophages³, synthesize immunoreactive corticotrophin (ir-ACTH) and endorphins. The ir-ACTH produced appears to be identical with authentic ACTH¹⁻⁶, while enough of the material has been produced in hypophysectomized mice infected with virus to demonstrate a steroidogenic response⁶. Because the production of ACTH by *in vivo* pituitary cells and by leukocytes is suppressed by dexamethasone both *in vitro*⁷ and *in vivo*⁶, suggesting⁸ that the production of ACTH and endorphins by leukocytes is indeed controlled, we have investigated the effects of corticotropin releasing-factor (CRF), which is known⁹ to regulate the pituitary production of both ACTH and β -endorphin. We now report that the production of ACTH and endorphins by leukocytes is indeed induced by synthetic CRF¹⁰ and, in turn, suppressed by dexamethasone, suggesting that, as in pituitary cells, the pro-opiomelanocortin (POMC) gene may be expressed and similarly controlled in leukocytes.

At the outset, human peripheral leukocytes were incubated in various concentrations of synthetic ovine CRF and then stained by immunofluorescence with antibody monospecific against ACTH (1-13 amide). The proportion of cells positive for ir-ACTH increased linearly with CRF concentrations between 0.7 and 70 nM (with background staining generally about 10%). The percentage of positive cells was generally at a maximum after 48 h, although positive cells were usually observed as early as 24 h (data not shown). The concentrations of CRF required to induce ir-ACTH in leukocytes were within the range of those required to induce production by primary⁹⁻¹² and transformed¹³ pituitary cells. The chief difference between the induction of ACTH from pituitary cells and leukocytes appears to be kinetic; while the former respond within minutes of stimulation, the latter respond only in hours, which may be explained by the requirement of *de novo* expression in leukocytes.

Because arginine-vasopressin (AVP) both has intrinsic CRF activity⁹ and also enhances the activity of CRF itself^{12,14-17} on pituitary cells, we also investigated the effects on leukocytes of incubation with AVP with or without CRF. At a concentration of 10 nM, AVP alone increases the production of cells positive for ir-ACTH to a degree equivalent to that by 0.7 nM CRF (see Table 1). Our observation that the presence of AVP (at the same concentration) also augments the response to CRF shows that, with leukocytes as with pituitary cells, the two hormones are similarly synergistic.

Accordingly, we set out to demonstrate that the antigen induced by CRF and detected by immunofluorescence is indeed a peptide resembling ACTH. To this end, we treated human peripheral leukocytes for various times with synthetic ovine CRF and AVP in the presence of tritiated amino acids so as to yield radio-labelled peptides. The ir-ACTH was purified by affinity chromatography on a Sepharose 4B column loaded with a monospecific antibody (1-13 amide) and then sized by gel filtration.

The radiolabelled ir-ACTH (see Fig. 1) has a relative

Table 1 Dose response for CRF induction of ir-ACTH

CRF (nM)	% ir-ACTH-positive cells No AVP	+ AVP (10 nM)
0	13 ± 4	30 ± 7
0.7	28 ± 4	50 ± 14
7.0	38 ± 11	65 ± 7
70	58 ± 11	90 ± 14

Human peripheral leukocytes were prepared on a Ficoll-Hypaque buoyant density gradient²¹ and resuspended in Eagle's MEM (supplemented with 2% fetal bovine serum) at 1×10^7 cells per ml. The lymphocytes were incubated with the indicated concentrations of synthetic ovine CRF (Boehringer Mannheim) and arginine vasopressin (AVP, Sigma) for 48 h. The lymphocytes were then washed, fixed with ethanol on coverslips, and stained by a previously described indirect immunofluorescence technique with antiserum made against synthetic ACTH (1-13 amide)¹. The results are expressed as the percentage of leukocytes staining positively $\pm$ the standard deviation between replicate coverslips.

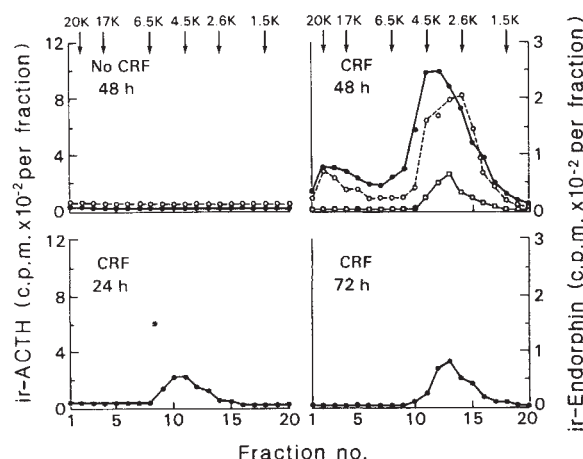


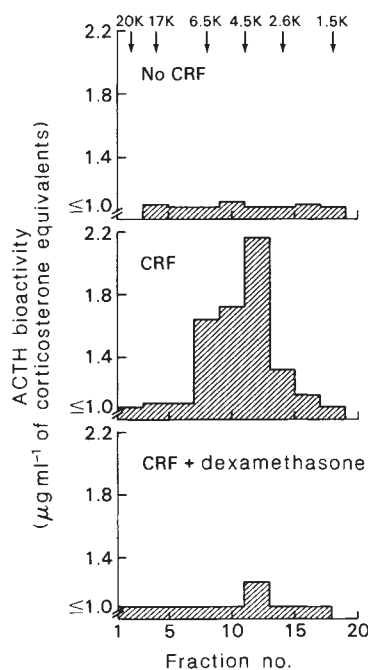
Fig. 1 Kinetics of production and molecular weight determination of CRF induced leukocyte-derived ir-ACTH and ir-endorphin. Ficoll-Hypaque prepared HPLs ($1 \cdot 10^7$ ml⁻¹) were induced for the indicated period of time with CRF (100 ng ml⁻¹) and AVP (100 ng ml⁻¹) in the presence of 1 μ Ci each of tritiated: glutamic acid (44.1 Ci mmol⁻¹), tyrosine (52.9 Ci mmol⁻¹), lysine (87 Ci mmol⁻¹), methionine (80 Ci mmol⁻¹), and histidine (12.8 Ci mmol⁻¹) purchased from NEN. One 48-h culture was also treated with dexamethasone (0.5 μ g ml⁻¹) CRF and AVP. The culture supernatant fluids were passed over an anti-ACTH (1-13 amide) or an anti- γ -endorphin antibody-Sepharose 4B affinity column (1, 4, 7) and eluted with 0.1 M glycine (pH2). A fraction of the radiolabelled material that bound to the affinity columns from each sample was analyzed by gel filtration on a 1 $\times$ 16 cm P-10 (Bio Rad, Richmond, CA) column. The column runs were eluted with 0.1 M phosphate buffered saline (pH 7.2) and quantitated by liquid scintillation spectroscopy (42% counting efficiency for ³H). Immunoreactive ACTH and endorphin are represented by closed and open circles respectively. Immunoreactive ACTH produced at 48 h in the presence of CRF, AVP and dexamethasone is represented by open squares.

molecular mass, M_r , corresponding to that of authentic ACTH (approximately 4,500). Our experiments show that ir-ACTH was evident after 24 h of treatment with CRF, was at a peak at 48 h and that it had declined by 72 h. In the absence of CRF, no radiolabelled ir-ACTH was detected in the culture fluids. The smaller peak of radiolabelled material (M_r in the range 17,000-20,000) may represent the high- M_r forms of ACTH which have been described previously¹⁸.

In other experiments (Fig. 1), we found that the presence of dexamethasone markedly reduced the amount of ir-ACTH produced by CRF in leukocyte cultures after 48 h. Moreover,

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Fig. 2 Steroidogenic activity of CRF-induced leukocyte-derived ir- β -ACTH. Fractions from the P-10 gel filtration column (Fig. 1) were diluted in F-10 medium and incubated with cultured Y-1 adrenal tumor cells (1–5) for 18 h. The cell culture supernatant fluids were then assayed for glucocorticoid production in a previously described radioimmunoassay for corticosterone (6).



neither the release of ir- β -ACTH nor the suppression of its production by dexamethasone is attributable to cell death; we found, by trypan blue exclusion measurements, that 95% of the cells in our cultures were viable at all times in the range covered by our experiments.

In order to determine the biological activity of the induced ir- β -ACTH, gel-filtration fractions were assayed for steroidogenic activity in cultured mouse adrenal tumour (Y-1) cells; we found that the peak of steroidogenesis is associated with the 4,500 dalton fraction (Fig. 2), but that samples from the control cultures (with no CRF) and from those treated with dexamethasone have no significant steroidogenic activity. By comparison with the activity of an ACTH standard, we conclude that treatment with CRF for 48 h induces the production of 650 ng of biologically active ACTH from 1×10^5 leukocytes.

We also investigated the possible production by leukocytes of other peptides derived from POMC by examining the radiolabelled culture fluids for immunoreactive endorphins. Fluids from 48 h treated and control (no CRF) cultures of peripheral human leukocytes were passed over a monospecific anti- β -endorphin Sepharose 4B column and the material retained was sized by gel filtration. The treated leukocytes, but not the controls, synthesized an immunoreactive radiolabelled endorphin of M_r approximately that of β -endorphin, or 3,500 (Fig. 1), but the synthesis was inhibited by dexamethasone (data not shown). The two series of experiments together show that CRF induces the production of both ir- β -ACTH and endorphins from peripheral human leukocytes.

These data strongly suggest that the POMC gene can be expressed in leukocytes, as it is in pituitary cells, and that it is similarly controlled. Our findings have both practical and theoretical implications. We have previously shown⁶ that virus-induced spleen ir- β -ACTH can replace pituitary ACTH, inducing steroidogenesis in hypophysectomized mice; that and our present data suggest that it may be possible to correct pituitary ACTH deficiencies by the administration of exogenous CRF, which may induce ir- β -ACTH from leukocytes. We also note that the occurrence of hormones and receptors which are common to both the immune and neuroendocrine systems may be a means by which the two systems communicate with each other, providing a regulatory circuit linking the hypothalamus and the immune system which may be coupled with the previously described⁶ lymphoid adrenal axis involving leukocyte ACTH and adrenal steroids. We suspect that the immune system may

in this way have a sensory function¹⁹, sending signals to the pituitary in response to the presence of somatic stimuli such as viruses, bacteria and tumours. Conversely, the central nervous system may send signals to the immune system by means of hypothalamic hormones such as CRF. These findings may thus provide a biochemical basis for the idea that states of mind may be reflected in states of the immune system²⁰.

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The β -subunit of follicle-stimulating hormone is deleted in patients with aniridia and Wilms' tumour, allowing a further definition of the WAGR locus

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One in 10,000 children develops Wilms' tumour, an embryonal malignancy of the kidney¹. Although most Wilms' tumours are sporadic, a genetic predisposition is associated with aniridia, genito-urinary malformations and mental retardation (the WAGR syndrome)². Patients with this syndrome typically exhibit constitutional deletions involving band p13 of one chromosome 11 homologue^{3,4}. It is likely that these deletions overlap a cluster of separate but closely linked genes that control the development of the kidney, iris and urogenital tract (the WAGR complex). A discrete aniridia locus, in particular, has been defined within this

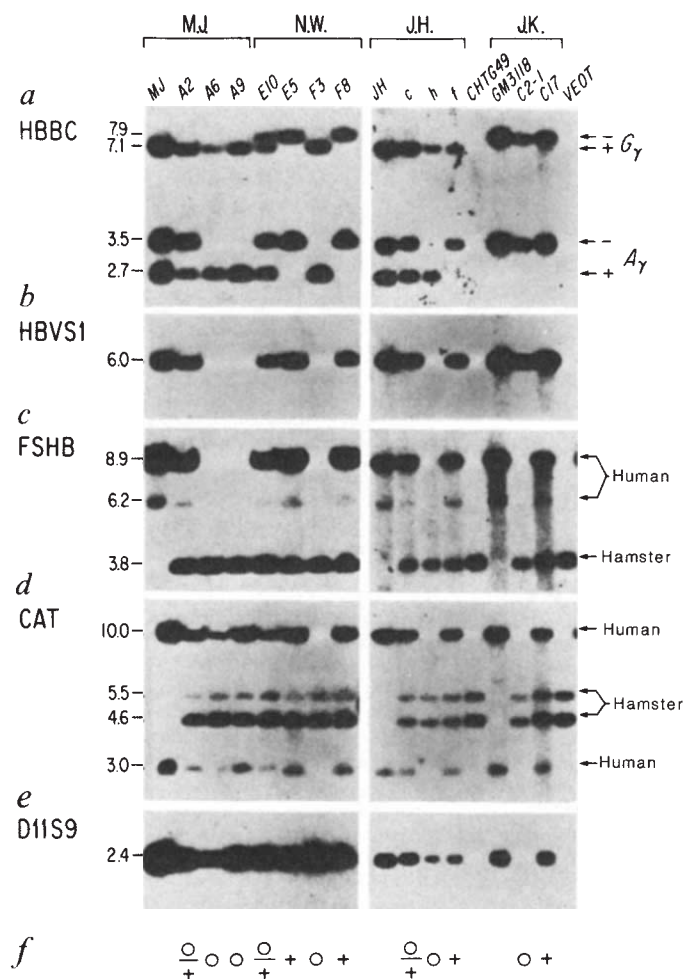


Fig. 1 Southern blot analysis of somatic cell hybrids from four patients with the WAGR syndrome. The panels show *Hind*III-digested DNA from hybrid clones and their parental human and hamster cell lines. They represent filters that were sequentially probed with different cloned DNA fragments. Each panel is a composite of two autoradiographs. The results show the segregation of chromosome 11 homologues in the hybrids, and the deletion of four markers in the patients. The size of each hybridizing fragment is given in kilobases to the left of the figure. Cell lines: M.J., an EBV-transformed lymphoblast culture obtained from patient M.J.; J.H. and GM3118, skin fibroblast cultures obtained from patients J.H. and J.K.; CHT649 and VEOT, Chinese hamster parental cell lines; A2, A6 and A9, hybrid clones derived from patient M.J.^{14,50}; E10, E5, F3 and F8, hybrid clones derived from patient N.W.^{14,50}; c, h and f, hybrid clones derived from patient J.H.; C2-1 and C17, hybrid clones derived from patient J.K.¹⁵. **a**, Nylon filters hybridized with the 2.7-kb *Eco*RI insert of p γ 2.7 (ref. 55). Fragments corresponding to the human γ and γ globin genes are indicated to the right of the figure. This probe reveals a RFLP in both fetal globin genes. In each case, alleles are determined by the presence (+) or absence (-) of a *Hind*III site within the second intron²¹. Because patients M.J., N.W. and J.H. are heterozygous at this locus, the chromosome 11 homologues present in their hybrid derivatives can be distinguished. **b**, Identical filters rehybridized with the 1.2-kb *Hind*III-*Sca*I insert of pS8-1 (ref. 26). **c**, The same filters rehybridized with the 1.4-kb *Pst*I insert of pFSH-1.4 (refs 24, 25). The human and cross-reacting hamster β -FSH bands are labelled. **d**, The same filters rehybridized with the 0.6-kb *Pst*I-*Ava*I fragment of cDNA clone pC24 (ref. 14). The human and cross-reacting hamster catalase bands are labelled. **e**, The same filters rehybridized with the 0.8-kb *Hind*III-*Sac*I insert of pES1-2, a subclone of recombinant phage H11-22 (ref. 22). **f**, The chromosome 11 content of hybrid cell lines, deduced from Southern blot analysis. Chromosomes 11 present in the hybrids are indicated as normal (+) or deleted (○). **Methods.** The selection of hybrid clones is detailed in Table 1 legend. Cultured cells were lysed in ice-cold buffer (0.5% Triton X-100, 50 mM HEPES pH 8.0, 5 mM MgCl₂, 50 mM NaCl, 100 g l⁻¹ sucrose), and their nuclei were recovered by centrifugation. Genomic DNA was isolated from the nuclear pellets as described previously²² and digested with a 3-4-fold excess of *Hind*III. The digested DNA (10 μ g per lane) was separated by electrophoresis through a horizontal 0.8% agarose gel and transferred to Zetabind (AMF-Cuno) nylon filters^{47,66}. Insert fragments from plasmid clones p γ 2.7, pS8-1, pFSH-1.4, pC24 and pES1-2 were isolated from low-melting-point agarose gels and radiolabelled to high specific activity with [α -³²P]dCTP (>10⁸ c.p.m. μ g⁻¹), using the Klenow fragment of *Escherichia coli* polymerase I and random primers⁵⁸. After transfer, filters were baked in a vacuum oven (80 °C, 2 h), pretreated for 1 h at 65 °C in 0.5% SDS 0.1×SSC (1×SSC=0.15 M NaCl, 0.015 M Na citrate) and prehybridized overnight. Filters were then hybridized for 2 days with 10⁶ c.p.m. ml⁻¹ radiolabelled probe DNA. Prehybridization and hybridization were at 42 °C in 50% formamide (Nucleic Acid Grade, EM Industries), 1 M NaCl, 50 mM Tris (pH 7.4), 6% dextran sulphate, 1×Denhardt's⁶⁹, 0.5% SDS and 1 mg ml⁻¹ autoclaved salmon sperm DNA. After hybridization, filters were washed three times in 0.1% SDS 1×SSC at 55 °C, and exposed to Kodak XAR-5 X-ray film at -70 °C for 3-4 days, using a Dupont Lightning-Plus intensifying screen. Bound probe was removed before rehybridization by bathing the filters in hybridization solution at 70 °C for 1 h.

chromosomal segment by a reciprocal translocation, transmitted through three generations, which interrupts 11p13 (ref. 5). In addition, the specific loss of chromosome 11p alleles in sporadic Wilms' tumours has been demonstrated, suggesting that the WAGR complex includes a recessive oncogene⁶⁻⁹, analogous to the retinoblastoma locus on chromosome 13 (ref. 10). In WAGR patients, the inherited 11p deletion is thought to represent the first of two events required for the initiation of a Wilms' tumour, as suggested by Knudson from epidemiological data¹¹. We have now isolated the deleted chromosomes 11 from four WAGR patients in hamster-human somatic cell hybrids, and have tested genomic DNA from the hybrids with chromosome 11-specific probes. We show that 4 of 31 markers are deleted in at least one patient, but that of these markers, only the gene encoding the β -subunit of follicle-stimulating hormone (*FSHB*) is deleted in all four patients. Our results demonstrate close physical linkage between *FSHB* and the WAGR locus, suggest a gene order for the four deleted markers and exclude other markers tested from this region. In hybrids prepared from a balanced translocation carrier with familial aniridia⁵, the four markers segregate into proximal and distal groups. The translocation breakpoint, which identifies the position of the aniridia gene on 11p, is immediately proximal to *FSHB*, in the interval between *FSHB* and the catalase gene.

Although numerous genes and arbitrary DNA segments have been assigned to chromosome 11p (ref. 12), few sequences have been mapped within the WAGR region. The best-characterized example, erythrocyte catalase, has been localized to 11p13 by correlating the level of catalase activity with the dosage of this chromosomal segment in mono- and trisomic individuals¹³. More recently, several investigators have confirmed this result, using complementary DNA clones that code for catalase as molecular probes¹⁴⁻¹⁷. To define additional DNA markers in this region, we analysed the chromosomes 11 of four WAGR patients whose deletions involve band p13. Cytogenetic findings for three of the patients have been reported previously^{18,19} (see Fig. 2). To identify DNA sequences deleted in each patient, we constructed somatic cell hybrids between the patients' primary somatic cells and a Chinese hamster cell line, as described in Table 1 legend. This allowed us to physically segregate the chromosomes 11 from each patient so that the individual homologues could be assayed separately. Several independent hybrid clones were isolated from each fusion, and those hybrids containing one or more chromosome 11 were detected by their expression of human lactate dehydrogenase A (*LDHA*)²⁰, a marker outside the deleted region, on the short arm¹².

To identify chromosome 11 homologues, we tested the hybrid

clones with DNA markers that exhibit significant restriction fragment length polymorphism (RFLP). At loci where a particular patient is heterozygous, hybrid derivatives segregating a single chromosome 11 homologue should carry only one allele. Figure 1a shows an example of this analysis, where genomic DNA from three sources—patients M.J., J.H. and J.K., representative hybrid clones and Chinese hamster parental cell lines—has been hybridized with $\gamma\gamma 2.7$, a human fetal globin-specific probe. This probe identifies two polymorphic *Hind*III sites in the β -haemoglobin gene cluster (*HBBC*), within the γ and δ genes²¹. The β -globin marker has been localized to 11p15 by *in situ* hybridization, meiotic linkage analysis and somatic cell genetic techniques¹². As shown, the hybridization pattern gives the genotype of each patient, and allows us to identify the chromosome 11 homologues present in each hybrid cell line. For example, patient J.H. is homozygous at the γ site (+/+), giving a 7.1-kilobase (kb) band, but he is heterozygous at the δ site (+/-), and so has both 2.7-kb and 3.5-kb bands. A comparison of hybrid clones derived from J.H. reveals one clone (h) with the 2.7-kb band, one clone (f) with the 3.5-kb band, and one clone (c) with both allelic bands. Therefore, hybrid clones h and f contain different chromosome 11 homologues, and clone c contains both. One hybrid cell line, either h or f, must carry only the del(11) homologue. Analysis of patients M.J., N.W. and J.K. and their hybrid derivatives shows similar patterns of segregation. In the case of patient M.J., however, only one chromosome 11 homologue has been isolated in a hybrid cell line. For patient N.W., total genomic DNA is not available, so the genotype has been reconstructed by the pattern of alleles in hybrid clones.

Table 1 summarizes the results obtained with these cell lines at multiple polymorphic loci on chromosome 11. The markers listed span both chromosome arms and are present in every hybrid tested. These data suggest that the structure of the patients' chromosomes 11 has been preserved in the hybrids, and that the markers listed lie outside the *WAGR* region. The pattern of segregation defines a haplotype for each homologue, but it does not indicate which haplotypes are associated with deletions. This assignment emerged from our analysis of four markers that are deleted (Figs 1b-e).

In Fig. 1e, we have probed the filters shown in Fig. 1a with pES1-2, an arbitrary genomic fragment designated *D11S9* (ref. 22). This marker is present in every hybrid tested except C2-1, which carries the del(11) homologue from patient J.K.¹⁵. In Fig. 1d, we have probed these filters with pC24, a cDNA clone encoding erythrocyte catalase (*CAT*)¹⁴. This probe recognizes two *Hind*III fragments in human DNA and cross-hybridizes with hamster DNA. Hybrid clones from three patients—N.W., J.H. and J.K.—lack the human catalase gene. As expected, these cell lines—F3, h and C2-1—each contain a single chromosome 11 homologue. In contrast, two clones from patient M.J.—A6 and A9—are positive for *CAT*, yet contain only the deleted chromosome 11 (see below). These data are consistent with the finding of Punnett *et al.*²³ that four out of five *WAGR* patients have decreased catalase activity.

In Fig. 1c these filters have been probed with pFSH-1.4, a 1.4-kb *Pst*I fragment containing the structural gene for β -follicle-stimulating hormone (*FSHB*)^{24,25}. Like *CAT*, this probe recognizes both human and hamster sequences. *FSHB*, which has been mapped to 11p11-pter (ref. 25), is absent in hybrid clones from all four patients, including M.J. As before, this deficiency is correlated with a particular chromosome 11 haplotype in each patient.

In Fig. 1b, filters were probed with pS8-1, a genomic fragment isolated from a hepatocellular carcinoma, flanking a hepatitis B viral (HBV) insertion (Fig. 1b)²⁶. This marker, designated *HBVS1*, has been localized to 11p13-p14 by *in situ* hybridization. In patients M.J., N.W. and J.H., the *HBVS1* marker is missing from one chromosome 11 homologue, but J.K. has this sequence on both homologues. Provided the four patients' deletions are

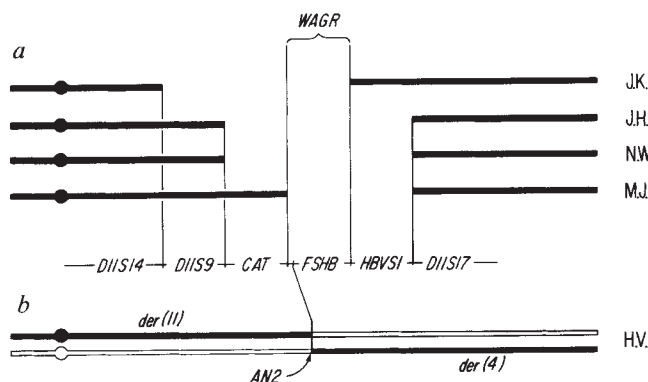


Fig. 2 Genetic map of the *WAGR* region based on five patients. **a**, Deleted chromosomes 11 of four *WAGR* patients. Depicted are the short arm and centromere of chromosome 11. The horizontal bars represent chromosomal segments that are retained in the affected homologues. In cytogenetic terms, the deletions in patients J.K. and J.H. are represented as del(11) (p13p11)¹⁸ and del(11) (p14.1p11.2) (R. Nussbaum, personal communication), respectively. Precise cytogenetic breakpoints have not been determined for patients N.W. or M.J.¹⁹. The four central markers—*D11S9*, *CAT*, *FSHB* and *HBVS1*—are deleted in at least one patient. Loci excluded from the *WAGR* region (Table 1) are represented by *D11S14* and *D11S17*, the nearest outside markers^{34,35}. The deleted markers define four discrete intervals in this region. Although seven are predicted from the four deletions ($=2n-1$, for n independent interstitial deletions), three intervals cannot be resolved by the present set of markers. **b**, Translocation chromosomes associated with familial aniridia. Both derivative chromosomes of patient H.V. are shown. Segments derived from chromosomes 11 and 4 are drawn as solid and open bars, respectively. The detailed karyotype, including G- and R-banded preparations, has been published previously⁵. The translocation, represented as t(4; 11)(q22; p13), serves to orient the deletion map with respect to the centromere. Its breakpoint on 11p lies between *CAT* and *FSHB*, and identifies the aniridia gene (*AN2*). The data presented do not specify the position of *FSHB* in relation to other genes in the *WAGR* complex (for example *WT*).

contiguous, *HBVS1* and *CAT* must flank both *FSHB* and the *WAGR* complex.

In the original hepatocellular tumour, the integrated HBV genome itself spanned a deletion of unknown size, exceeding 13 kb. The proximity of this integration site to 11p13 led Rogler *et al.*²⁶ to propose that the HBV-associated deletion includes the Wilms' tumour locus, and that the loss of one allele at this locus on viral insertion may have contributed to the malignant progression of this liver tumour. When tested, a second probe, which had been isolated from the opposite side of the deletion, was also present in both J.K. derivatives (data not shown). Because J.K.'s deletion spans the *WAGR* complex, these data suggest that the HBV-associated deletion does not overlap the Wilms' tumour gene. The alternative explanation, that one deletion is contained inside the other, is inconsistent with results obtained from the other patients and markers.

Figure 1f summarizes the chromosome 11 content of each hybrid as determined from our analysis of haplotypes and deleted markers. By comparing the extent of the deletions, we have ordered four markers in relation to the *WAGR* locus. The resulting map (Fig. 2a) reflects our assumption that the deletions are contiguous. Two of the patients—J.H. and N.W.—lack the same set of markers. Presumably, the corresponding end points of their deletions could be resolved by testing additional markers in this region. To orient this map with respect to the centromere, we have used a reciprocal translocation that breaks chromosome 11 at band p13, and disrupts the aniridia gene.

Bilateral aniridia is a rare autosomal dominant trait characterized by rudimentary development of the iris²⁷⁻²⁹. Mutations in

Table 1 Chromosome 11 haplotypes of four WAGR patients

Marker	Probe	Enzyme	M.J.		N.W.		J.H.		J.K.		Ref.
	Short arm		del	nor	del	nor	del	nor	del	nor	
<i>HRAS1</i>	pEJ6.6	<i>Bam</i> HI	8.0	8.0	6.6	6.6	+	+	6.6	8.0	51
<i>INS/IGF2</i>	phins321	<i>Sac</i> I	5.8	6.0	5.8	7.6			6.4	6.4	52
<i>CPSD</i>	pCD1.1		+	+	+	+					53
<i>D11S1</i>	pCS1		+	+	+	+					22
<i>D11S26</i>	p α -1b		+	+	+	+					36
<i>D11S25</i>	p γ -6		+	+	+	+					36
<i>D11S12</i>	pADJ762		+	+	+	+					54
<i>HBG2</i>	p γ 2.7	<i>Hind</i> III	7.1	7.1	7.1	7.9	7.1	7.1	7.1	7.1	55
<i>HBG1</i>	p γ 2.7	<i>Hind</i> III	2.7	3.5	2.7	3.5	2.7	3.5	3.5	3.5	55
	pJ1.1		+	+	+	+					36
<i>D11S20</i>	pJ14.1	<i>Pst</i> I	1.3	1.3	4.0	1.3					36
<i>CALC1</i>	pTT42	<i>Taq</i> I	+	+	+	+			8.0	6.5	56
<i>PTH</i>	pPTH2.5	<i>Pst</i> I	2.2	2.2	2.7	2.7					57
<i>D11S21</i>	pE40-4A		+	+	+	+			+	+	58
<i>LDHA</i>	activity		+	+	+	+	+	+	+	+	20
<i>D11S18</i>	pJ24.2	<i>Sac</i> I	12.0	4.2	12.0	4.2			+	+	36
<i>D11S17</i>	pJ19.4	<i>Bam</i> HI	21.0	21.0	13.0	21.0	+	+	+	+	36
<i>D11S14</i>	pDS1		+	+	+	+			+	+	22
	pJ17.3.5	<i>Eco</i> RI	3.1	3.8	3.8	3.8					36
Long arm											
<i>PGA</i>	phpep14-21		+	+	+	+					59
<i>PYGM</i>	pMH11		+	+	+	+					60
<i>INT2</i>	pSS6		+	+	+	+					61
<i>ASSP13</i>	pAS-1		+	+	+	+					62
<i>D11S24</i>	pE4b-TGH2	<i>Bam</i> HI	15.0	12.0	12.0	12.0					58
<i>D11S23</i>	pE45-RL4		+	+	+	+					58
<i>PC</i>	pPhC1		+	+	+	+					63
<i>APOA1</i>	λ ApoA1-6	<i>Sac</i> I	3.0	4.3	4.3	4.3	+	+	+	+	64
<i>T3D</i>	pPGBS9		+	+	+	+					65

Chromosome 11 haplotypes were determined by the segregation of RFLP alleles in somatic cell hybrids. Where RFLPs have been scored, approximate sizes of the relevant hybridizing fragments are given in kilobases. Otherwise, markers tested are indicated as present (+). Haplotypes were identified as belonging to the deleted (del) or normal (nor) homologue by the absence of various markers from particular cell lines (Fig. 1). The status of markers on M.J.'s normal chromosome 11 was inferred from hybrid A2, which contains both homologues. For each patient, the results represent our analysis of several independent hybrid clones.

Methods: Skin fibroblasts from patient J.H. were fused with thioguanine-resistant CHTG49 hamster cells⁴⁸, using polyethylene glycol 1000 (Koch-Light), and hybrid clones were selected in hypoxanthine-aminopterin-thymidine (HAT) medium supplemented with 10% fetal calf serum and 1 μ M ouabain⁴⁹. Hybrid colonies were expanded and recloned in HAT medium, and 10–15 recloned cell lines were studied in detail. Somatic cell hybrids derived from the three other patients (N.W., M.J. and J.K.) have been described previously^{14,15,50}; hybrids representing N.W. and M.J. were obtained by fusing white blood cells with E36 hamster cells, and hybrids representing J.K. were obtained by fusing fibroblast culture GM3118 (NIGMS Human Genetic Mutant Cell Repository, Camden, New Jersey) with VEO-3 hamster cells. For each marker, genomic DNA from hybrid cultures was cleaved with the restriction endonuclease shown and analysed by Southern blotting as described in Fig. 1 legend, using the indicated radiolabelled probe. Genetic symbols¹²: *HRAS1* (c-Ha-ras-1 oncogene), *INS* (insulin), *IGF2* (insulin-like growth factor 2), *CPSD* (cathepsin D), *HBG1* (γ fetal haemoglobin), *HBG2* (α fetal haemoglobin), *CALC1* (calcitonin), *PTH* (parathyroid hormone), *LDHA* (lactate dehydrogenase A), *PGA* (pepsinogen A), *PYGM* (muscle phosphorylase), *INT2* (human homologue of a murine mammary tumour virus integration site), *ASSP13* (an argininosuccinate synthetase pseudogene), *PC* (pyruvate carboxylase), *APOA1* (apolipoprotein A1), *T3D* (T3- δ subunit of the T3-T-cell receptor), *CEN* (centromere), *TEL* (telomere).

at least two separate genes can produce this disorder. One gene (*AN1*) has been assigned to chromosome 2p by linkage to the acid phosphatase locus (*ACPI*)³⁰ and by the phenotype of a deletion carrier³¹; the second (*AN2*) forms part of the *WAGR* complex on chromosome 11p, as described above. To map the *AN2* gene on 11p, we have analysed a reciprocal translocation that is associated with familial aniridia⁵, and which has recombined the short arm of chromosome 11 with the long arm of chromosome 4. As described by Simola *et al.*⁵, the grandfather, mother and daughter in this family have bilateral aniridia, but have normal levels of blood catalase and no evidence of genitourinary defects or Wilms' tumour. All affected family members carry the translocation t(4; 11)(q22; p13). We fused a lymphoblastoid cell line, established from the affected grandfather (patient H.V.), with mouse thymidine kinase-negative L-cells (Ltk⁻), and isolated somatic cell hybrid clones as described elsewhere³². Two clones were identified that contain the derivative human chromosomes in the absence of a normal human chromosome 11. Hybrid LHV-3 contains the der(11) chromosome, while hybrid LHV-1 contains both der(11) and der(4) chromosomes (Fig. 2b).

To confirm these cytogenetic findings, we tested genomic DNA isolated from the hybrid clones with chromosome 11-specific probes. Table 2 summarizes the results for 10 genetic markers. Two patterns of segregation are evident in the hybrids. Markers present in LHV-1 but absent in LHV-3 are carried on the der(4) chromosome and are distal to the translocation breakpoint. Six markers—*HRAS1*, *CALC1*, *LDHA*, *D11S17*, *HBVS1* and *FSHB*—segregate with the der(4) chromosome. Markers

present in both LHV-1 and LHV-3 are carried on the der(11) chromosome and are proximal to the breakpoint. Recombinant probe p α -11 illustrates the segregation of proximal markers; it detects a family of α -satellite repetitive DNA specific for the centromeric region of chromosome 11 (ref. 33). When probed with p α -11, genomic DNA of both hybrids show hybridizing *Xba*I fragments characteristic of the human chromosome 11 centromeric region (data not shown). Three short-arm markers—*D11S14*, *D11S9* and *CAT*—and the long-arm marker *INT2* are present in both hybrids, as part of the der(11) chromosome. At polymorphic sites identified by two of these loci (*CAT* and *INT2*), patient H.V. is heterozygous; as expected, only one allele is present in the hybrids, a result consistent with the absence of the normal chromosome 11.

We have positioned the aniridia translocation breakpoint on the *WAGR* deletion map according to these data (Fig. 2b). Two of the markers deleted in *WAGR* patients are proximal to the breakpoint (*D11S9* and *CAT*) and two are distal (*FSHB* and *HBVS1*). In addition, markers excluded from the *WAGR* region (Table 1) are separated by the translocation into proximal and distal groups. We conclude that the break in 11p has occurred between the catalase and β -FSH genes, resulting in the translocation of *FSHB*, *HBVS1*, *D11S17*, *LDHA*, *CALC1* and *HRAS1* to chromosome 4. The segregation of *LDHA* with the telomeric portion of 11p further supports our conclusion^{32,34} that *LDHA* is distal to the catalase gene, and not at 11p12 as previously reported¹².

Together, these data suggest the following gene order for chromosome 11p: *CEN-D11S14-D11S9-CAT-FSHB-HBVS1-*

Table 2 Translocation of chromosome 11 markers in familial aniridia

Marker	Probe	Enzyme	Chromosome			Ref.
			der(4)*	der(11)†	nor(11)	
<i>HRAS1</i>	pEJ6.6		+	—	+	51
<i>CALC1</i>	pTT42		+	—	+	56
<i>LDHA</i>	activity		+	—	+	20
<i>D11S17</i>	pJ19.4		+	—	+	36
<i>HBVS1</i>	pS8-1		+	—	+	26
<i>FSHB</i>	pFSH-1.4		+	—	+	25
<i>CAT</i>	pCAT-41	<i>KpnI</i>	—	8.0, 3.0	11.0	15
<i>D11S9</i>	pES1-2		—	+	+	22
<i>D11S14</i>	pDS1		—	+	+	22
	p α -11‡		—	+	+	33
<i>INT2</i> §	pSS6	<i>BamHI</i>	—	8.4	5.6, 2.8	61

Reciprocal translocation chromosomes from H.V. (patient 3 in ref. 5) were segregated in somatic cell hybrids. Genomic DNA from the resulting hybrid clones was tested for specific human sequences by Southern blotting⁶⁶, using the indicated radiolabelled probes. At polymorphic loci where H.V. is heterozygous, the restriction endonuclease used is shown, and the sizes of the relevant hybridizing fragments are given in kilobases. Otherwise, markers tested are indicated as present (+) or absent (—). The presence of human *LDHA* was determined by cellophore electrophoresis²⁰. Hybrids were formed by fusing a lymphoblastoid cell line established from H.V. with mouse Ltk⁺ cells, as described elsewhere³². The results shown represent our analysis of two hybrid clones, LHV-3, containing the der(11) chromosome, and LHV-1, containing both der(11) and der(4) chromosomes. In these clones, the presence of the derivative human chromosomes in the absence of a normal human chromosome 11 was determined by detailed karyotyping. At least 15 metaphase spreads were examined at the time of DNA preparation, following trypsin-Giemsa banding⁶⁷. Genetic symbols are defined in the text and Table 1 legend.

* Derivative chromosome 4 (4pter>4q22::11p13>11pter). These data are inferred from hybrid LHV-1.

† Derivative chromosome 11 (11qter>11p13::4q22>4qter).

‡ Detects centromeric α -satellite DNA³³. A 0.85-kb *XbaI* band, specific for human chromosome 11, was scored.

§ The *INT2* marker has been mapped to 11q13 (ref. 61). All other markers tested are located on the short arm.

(*D11S17*, *LDHA*, *CALC1*, *HRAS1*)-TEL). This order is consistent with our analysis of other reciprocal translocations (in preparation and ref. 32) and of multi-point meiotic linkage^{35,36}. In separate reports (in preparation and ref. 34), we have extended the map shown in Fig. 2 using 11p deletions selected *in vitro*; our results support the *WAGR* deletion series and give an order to markers that have been excluded from the *WAGR* region (Table 1).

The observation that all of the markers tested are present on either the der(11) or der(4) chromosomes suggests that the aniridia translocation has not been accompanied by a large deletion of DNA. The simplest explanation is that the translocation has disrupted or deleted a relatively small area of 11p containing the aniridia gene (*AN2*) but not the Wilms' tumour gene (*WT*). If this interpretation is correct, and no complex rearrangement has accompanied the translocation, the aniridia gene must lie at the breakpoint, in the interval between *CAT* and *FSHB*. This configuration, represented as CEN-CAT-AN2-FSHB-TEL, conforms with the findings of Turleau *et al.*³⁷ and van Heyningen *et al.*¹⁷ which suggest *CAT* is proximal to *WAGR*, but it differs from the orientation proposed by Narahara *et al.*³⁸.

The exact position of the Wilms' tumour gene is less clear. According to our results, *WT* maps between *CAT* and *HBVS1*, in one of three sub-intervals demarcated by the *AN2* and *FSHB* genes (Fig. 2). If *WT* were distal to *FSHB*, giving the order CAT-AN2-FSHB-WT-HBVS1, then the *WAGR* complex would encompass *FSHB*. This arrangement is especially intriguing, since it raises the possibility that the genito-urinary anomalies seen in *WAGR* patients are caused by a deficiency of β -FSH during embryonic development.

An alternative placement of *WT*, proximal to *AN2*, has been suggested by Turleau *et al.*³⁷. These authors describe a patient with a visible deletion of 11p13, reduced catalase activity and Wilms' tumour, but with no evidence of aniridia. Of the four

WAGR stigmata, aniridia has the highest penetrance^{39,40}, particularly when patients at risk are examined thoroughly, so that subtle iris defects can be detected^{28,29}. These observations suggest that *CAT* and *WT*, but not *AN2*, are deleted in this patient. If this interpretation is correct, and his deletion spares the β -FSH gene, then the order *CAT*-*WT*-*AN2*-*FSHB* is most likely.

Although the chromosomal rearrangements we have examined are visible in banded karyotypes, the map we present does not depend on cytogenetic data. However, we note that breakpoints carried by patients J.K. and H.V. immediately flank *FSHB* and occur in band p13. These data are consistent with the assignment of *CAT* to 11p13 (ref. 12) and *HBVS1* to 11p13-p14 (ref. 26), and they localize *FSHB* to band 11p13. This segment includes the *WAGR* gene complex and represents approximately 0.1% of the total human genome.

Several closely linked markers bracket the *WAGR* complex (Fig. 2). The nearest marker, *FSHB*, maps either within or distal to *WAGR*. From standard ideograms⁴¹, we estimate that the segment containing *CAT*, *FSHB* and *WAGR* spans 3,000–6,000 kb, the size of an average prokaryotic genome. As the density of markers increases in this interval, the distance separating probes should soon approach the range amenable to restriction mapping using pulsed-field gels⁴², and to molecular analysis by so-called 'jumping' techniques⁴³. In these studies, cell lines derived from patients with apparent microdeletions²⁹ and DNA samples from Wilms' tumours should prove especially useful. Despite the close physical linkage between *FSHB* and the Wilms' tumour gene, our attempts to show homozygous loss of *FSHB* in sporadic Wilms' tumours have been unsuccessful (T.G. and D.E.H., unpublished observations).

By segregating individual chromosome 11 homologues, we have gauged in molecular terms cytogenetic lesions associated with familial aniridia and the *WAGR* syndrome. The hybrid panel we have developed can be used to quickly locate chromosome 11-specific markers in the *WAGR* region. Our approach should be readily applicable to other disorders that are linked to autosomal deletions, including bilateral retinoblastoma⁴⁴, and the Praeder-Willi⁴⁵, cri du chat⁴⁶ and Wolf-Hirschhorn⁴⁷ syndromes.

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Ethanol production from sugars derived from plant biomass by a novel fungus

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The concept of transforming renewable plant biomass, and in particular organic waste, to fuels or chemicals continues to attract attention as nonrenewable fossil fuel reserves decrease^{1,2}. Cellulose and hemicellulose, which comprise more than 70% of plant biomass and are the two most abundant organic compounds in the biosphere, occur in the wastes from agriculture and forestry as well as from vegetable and fruit processing and municipal waste treatment. Recycling of these wastes to useful products helps to prevent pollution. After enzymatic or acid hydrolysis, plant biomass yields mostly cellobiose, xylose and glucose together with mannose, galactose and arabinose. Microorganisms which can ferment such a wide range of sugars, at high concentrations, into a high concentration of ethanol have not been reported previously. Here we report on a novel fungus, *Paecilomyces* sp. NF1, which is capable of fermenting all of the major sugars derived from hydrolysis of plant biomass to ethanol. In xylose fermentation, the ethanol yield of this fungus remained almost unchanged when the concentration of xylose was increased from 20 to 200 g l⁻¹. The fungus also produced good yields of ethanol from glucose, fructose, cellobiose, arabinose, galactose, mannose and ribose, as well as from the simultaneous saccharification and fermentation (SSF) of cellulose with hemicellulose, and from acid hydrolysates of plant biomass.

Paecilomyces sp. NF1 (ATCC 20766) was isolated from compost soil from Denver, Colorado. The medium used for isolation contained (g l⁻¹): yeast extract 3, yeast nitrogen base (Difco) 4, peptone 3, xylose 2 and agar 20 (for plates). The pH of the medium was adjusted to 2.5 with dilute hydrochloric acid. The fungus was isolated at 30 °C by the dilution plate method³. The composition of the fermentation medium was (g l⁻¹): yeast

Table 1 Maximum ethanol yields (g per g of sugar in medium) from sugar fermentation by *Paecilomyces* sp. NF1

Sugar	Sugar concentration (g l ⁻¹)	Ethanol production (g l ⁻¹)	Ethanol yield (g per g of sugar in medium)	Time for completion* (days)
D-Cellobiose	100	40.2	0.40	7
D-Fructose	100	39.9	0.40	7
D-Glucose	100	40.9	0.41	7
D-Galactose	100	40.4	0.40	7
D-Mannose	100	38.1	0.38	7
Starch	100	39.8	0.40	7
Xylose	100	39.8	0.40	7
L-Arabinose	50	13.8	0.28	4
Lactose	50	14.1	0.28	4
Maltose	50	21.1	0.42	4
D-Ribose	50	10.2	0.20	4

Fermentation was carried out at 30 °C in anaerobic tubes, shaken at 150 r.p.m. Initial pH was 5.0; initial cell density was 6.0 g l⁻¹.

* Number of days taken to completely ferment the individual sugar, as analysed by HPLC and gas chromatography. Higher sugar concentrations did not result in higher ethanol yields.

extract 1.5, KH₂PO₄ 1.0, K₂HPO₄ 1.0, NaCl 0.5, KNO₃ 2.5, MgSO₄·7H₂O 0.5, CaCl₂ 0.1 and test sugars at the desired concentrations. Tests showed that the fungus would not grow under anaerobic conditions, and would not produce ethanol under aerobic conditions, therefore the fungus was grown under aerobic conditions, and the mycelium was then placed in sealed Bellco tubes containing only a small volume of air (semi-anaerobic conditions). Note that the fungus can tolerate temperatures of up to 43 °C. *Paecilomyces* sp. NF1 can ferment a wide range of substrates, including glucose, galactose, fructose, mannose, maltose, soluble starch, xylose, arabinose, ribose and cellobiose (Table 1); sucrose and inulin yield only small amounts of ethanol. The fungus produced essentially the same amount of ethanol when the pH of the initial medium was varied from 2.2 to 7.0, and at either 30 °C or 37 °C. The ethanol yield from xylose fermentation by this fungus remained almost unchanged when the xylose concentration was increased from 15 to 200 g l⁻¹ (or 0.38 to 0.39 g per g of xylose)—that is, 75% of the theoretical yield. For a xylose concentration of 200 g l⁻¹, the final concentration of ethanol was 73 g l⁻¹ (Fig. 1, Table 2); this is the highest reported yield of ethanol from a xylose fermentation. These data

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Table 2 Ethanol production by various xylose-fermenting microorganisms

	Initial D-xylose concentration (g l ⁻¹)	Maximum ethanol concentration (g l ⁻¹)	Ethanol yield* (g l ⁻¹)	Ref.
<i>Candida tropicalis</i> ATCC 1369	75	8.28	0.11	8
<i>Candida</i> XF 217	50	21.0	0.42	9
<i>Candida shehatae</i> CSIR-Y492	50	18.0	0.36	10
<i>Candida shehatae</i> CSIR-Y492	90	26.2	0.29	11
<i>Fusarium oxysporum</i> VTT-D-80134	50	25.0	0.50	12
<i>Fusarium</i> F5†	50	25.0	0.50	12
	100	32.0	0.32	13
	150	43.0	0.29	
	200	40.0	0.20	
<i>Kluyveromyces</i> <i>marxianus</i> SUB-80-S	20	5.60	0.28	14
<i>Kluyveromyces</i> <i>cellobiovorus</i>	100	30.0	0.30	15
<i>Pachysolen</i> <i>tannophilus</i> NRRL-Y2460	20	5.30	0.26	16
<i>Pachysolen</i> <i>tannophilus</i> NRRL-Y2460	50	15.0	0.30	17
	115	27.6	0.24	
	158	33.2	0.21	
<i>Pachysolen</i> <i>tannophilus</i> RL-171†	80	21.0	0.26	18
	100	23.5	0.24	
	120	20.0	0.17	
	160	15.5	0.10	
	200	11.7	0.06	
<i>Paecilomyces</i> sp. NF1 ATCC-20766	50	19.7	0.39	Present work
	80	31.5	0.39	
	100	39.8	0.39	
	150	59.7	0.39	
	200	73.5	0.38	

* Ethanol yield: g ethanol produced per g xylose consumed.

† Estimated from the published data.

demonstrated that this fungus can tolerate high concentrations of both xylose and ethanol. *Paecilomyces* sp. NF1 produced high-purity ethanol from 200 g l⁻¹ of xylose. At the end of fermentation, as analysed by HPLC and gas chromatography, only trace amounts of other chemicals were detected: xylose (0.00 g l⁻¹), xylitol (0.45 g l⁻¹), acetate (0.45 g l⁻¹) and *n*-butyrate (0.04 g l⁻¹). Table 2 shows a comparison of ethanol production from xylose by *Paecilomyces* sp. NF1 with that of other organisms known to ferment xylose.

This fungus can produce nearly 60 g l⁻¹ ethanol from simultaneous saccharification and fermentation of either 150 g l⁻¹ cellulose or 100 g l⁻¹ cellulose with 50 g l⁻¹ hemicellulose (as represented by xylan). The latter result, illustrated in Fig. 1, represents the first report of SSF of both cellulose and hemicellulose to produce ethanol. Among the xylose-fermenting yeasts, only *Kluyveromyces cellobiovorus* is able to convert cellobiose into ethanol; however, the ethanol yield of this yeast was found to decrease when the cellobiose concentration was higher than 40 g l⁻¹. For example, it produced 20 g l⁻¹ ethanol from 40 g l⁻¹ cellobiose, but produced only 21 g l⁻¹ ethanol from 60 g l⁻¹ cellobiose⁴. The fungus *Paecilomyces* sp. NF1 also used all the sugars (30 g l⁻¹) from an acid hydrolysate of wheat straw to produce 12.5 g l⁻¹ ethanol, demonstrating that the fungus is not affected by toxic substances in hydrolysates, as are other fermenting microorganisms⁴⁻⁷.

Thus, *Paecilomyces* sp. NF1 has several unique characteristics with regard to ethanol fermentation. It can produce high concentrations of ethanol from the simultaneous saccharification and fermentation of celluloses derived from plant biomass. We believe that this fungus is a good candidate for use in the industrial conversion of plant biomass wastes into ethanol.

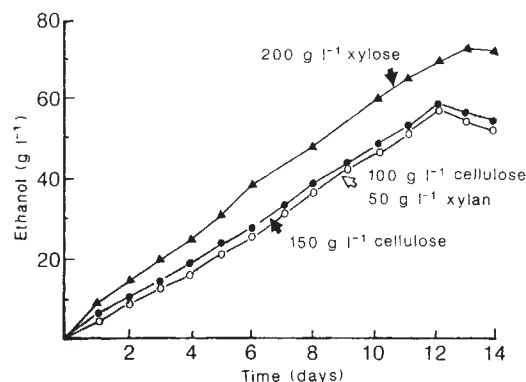


Fig. 1 Ethanol production from xylose (Δ) and simultaneous saccharification and fermentation (SSF) of cellulose ($\bullet$) or cellulose with xylan ($\circ$) by *Paecilomyces* sp. NF1. Initial pH 5.0, temperature 30 °C. The initial cell density was 6.0 g l⁻¹ for xylose fermentation and 4.5 g l⁻¹ for SSF. No increase in cell density occurred during the fermentation. In SSF, the cellulose used was Solka Floc BW200 and cellulase (from *Trichoderma reesei*) was added to a concentration equivalent to 8% of the cellulose; xylan was prepared from larch wood and hemicellulase (from *Aspergillus niger*) was used at a concentration of 8% of the xylan.

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Erratum

Mechanosensitivity of mammalian auditory hair cells *in vitro*

I. J. Russel, G. P. Richardson & A. R. Cody

Nature **321**, 517-519 (1986).

The first line in this letter should read 'Intracellular responses recorded *in vivo* from the cochleas ...', not '*in vitro*'.

Corrigendum

NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones

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